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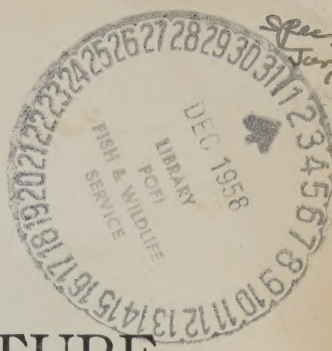

MEMOIRS
OF THE
COLLEGE OF AGRICULTURE
KYOTO UNIVERSIY

June 1958

(Fisheries Series Special Number)

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MEMOIRS OF THE COLLEGE OF AGRICULTURE

This special number has been published in commemoration of the 10th anniversary of the founding our Department of Fisheries and for our congratulating ourselves on the establishment of Misaki Marine Biological Institute.

Members of the Department

Director

Masao KIMATA

Professors

Wataru SIMIDU, D. Agr.	Chemistry
Tasae KAWAKAMI, D. Agr.	Physics
Masao KIMATA, D. Agr.	Microbiology
Kiyomatsu MATSUBARA, D. Sc.	Zoology

Assistant Professors

Saburô UENO	Chemistry
Hiroshi FUJITA, D. Sc.	Physics
Akira KAWAI	Microbiology
Yûichi YONEDA, D. Sc.	Botany

Instructor

Akira OCHIAI	Zoology

Members of the Institute

Director

Kiyomatsu MATSUBARA

Professors

Wataru SIMIDU	Tasae KAWAKAMI
Masao KIMATA	Kiyomatsu MATSUBARA

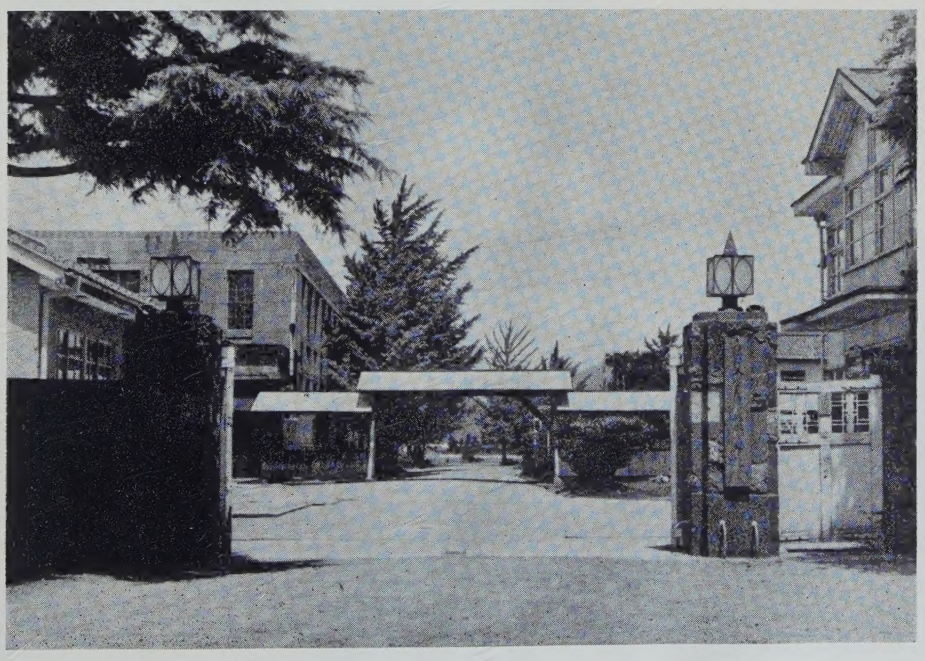
Editors

Assistant Professor	Hiroshi FUJITA
Assistant Professor	Akira KAWAI

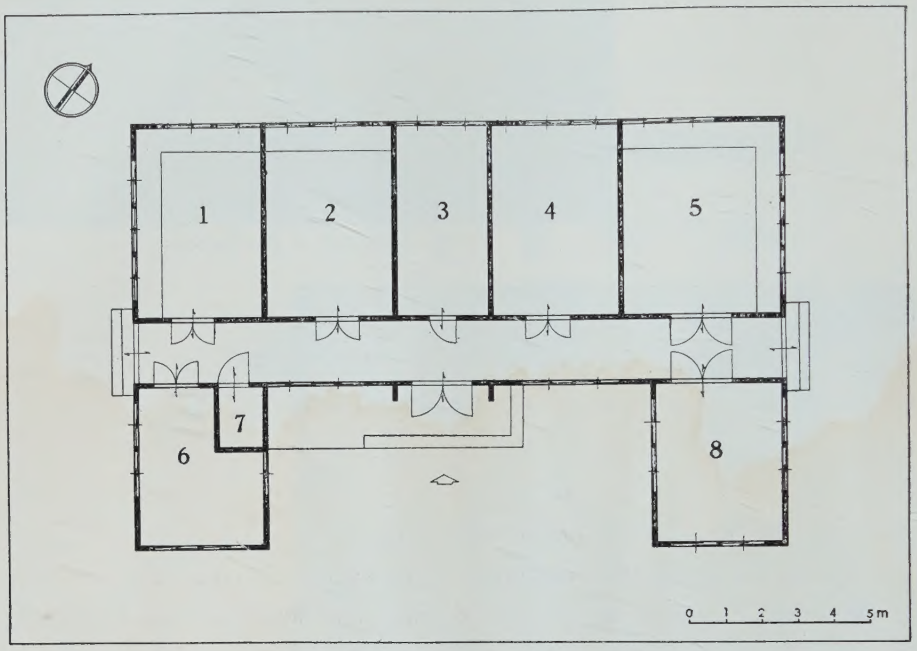
Acknowledgment

In our publishing this special number of ours the Nankai Electric Railway Co., Ltd. gave liberally to us. We wish to express our appreciation of the Company's support.

General view of the Department



Front of the Department



Ground-plan of the Institute

1. Laboratory of biology
2. Laboratory of biology
3. Superintendent's room
5. Laboratory of biochemistry
6. Implement room
7. Dark room
8. Specimen-room

History and Organization of the Department of Fisheries and the Misaki Marine Biological Institute

Department of Fisheries

Our Department of Fisheries was established as one of the departments of the Faculty of Agriculture of Kyoto University in May, 1947. It is aimed at the promotion of fishery science and further development of fishery industry.

It the Department are included four chairs: in the 1st chair industrial chemistry of marine products is mainly studied; in the 2nd chair, the theory of fishing gears and fishing methods, and industrial physics of fishery products; in the 3rd chair, microbiology of fishery products and peculiarities of marine microbiology; and the 4th chair, biology and management of aquatic animals and plants. Students are required to complete the whole course of study.

Our Department has been built at Nagahama, Maizuru in Kyoto prefecture, far away from the other departments of the Faculty of Agriculture, but is the only educational and research means of the University facing the Japan sea, the cultivation of the fishery resources in the same sea being mainly aimed at. The Department with a site of 15,296 *tsubo* (50,569.13m^2) has in it laboratories, lecture-rooms, a library, the office and a dormitory which cover an area of 1,669 *tsubo* (5,517m^2). Facing on the Bay of Maizuru with a calm sea, rich in marine animals and plants, the Department enables us to collect them at any time and conduct investigations on the sea, which cannot be expected of any other department of fisheries of an university. Some fishing grounds for the yellow tail, sardines and some other fishes important to the people are found near the site of our Department, enabling us to study aquatic resources and fishing condition whenever we wish. All these are characteristic of our Department of Fisheries. Moreover, the department is equipped with canning and refrigerating apparatuses, which afford us many oppotunities for practising and studying how to make marine products.

But we have to bear it in mind that our Department was founded in those hard times soon after Japan's defeat in the late war, when the supply of goods was very difficult, and that we were confronted with many difficulties which we had never encountered before. But hearty and intelligent co-

operation between the professors and the students since then for the betterment of the Department has come to bear fruit, and we have succeeded in making ours a department of fisheries so prominent as can hardly be seen in any other university of our country. As of the end of March, 1958 our Department turned out 151 boys, 16 who had completed the master course and 2 others who had completed the doctorate course. Those who are concerned in our Department have got 309 theses made public and 6 books published, some of which have been found to be valuable contributions to fisheries. Three of our staff have been awarded prizes by the Japanese Society of Scientific Fisheries (*Nippon Suisan Gakkai*) in consideration of their scientific services.

In May, 1957 we splendidly celebrated the 10th anniversary of the founding of our Department of Fisheries and a lecture meeting was held in commemoration of the occasion.

Misaki Marine Biological Institute

The Institute was established by the Nankai Electric Railway Co., Ltd. on March 20, 1958 for the purpose of contributing to the development of biological, and fishery, science.

It has been put free of change at the disposal of the Faculty of Agriculture of Kyoto University through the good offices of the same Company, and is kept open to the public all through the year.

In having the Institute at our own disposal, we have obtained facilities also for studying the sea different from the Japan Sea on which our Department looks out, for the Institute is located at Tannawa, Misaki, Sennan-gun, Osaka prefecture, facing the Inland Sea of Seto. As shown in the plane figure, the Institute covers an area of 50 *tsubo* (5,517 m^2) and is composed of the superintendent's room, a library, a specimen-room, an implement room and a dark room besides three laboratories.

List of Publications from the Department of Fisheries, Faculty of Agriculture, Kyoto University

(during the period 1947–1957)

The activities of members of our staff in their research toward the advancement of fishery science and technology during the past ten years since the foundation of the Department may be best illustrated by presenting a complete list of contributions they made for this period. Such a list has been compiled with generous cooperation of all the staff members, and is presented below. Papers (or books) published from particular laboratories of the Department are arranged chronologically, with index number, author's name, full title of article, journal's name, volume number (with issue number, when necessary), and initial and final pages in the order. Index numbers with an asterisk indicate that those papers were published in Japanese, generally accompanied with a brief synopsis in European languages. All other are written exclusively in English. Reprints of the listed articles may be obtained by writing directly to respective authors. It is hoped that this list of publications will serve investigators of other institutions to appreciate the high activities of our staff members in both fundamental and practical researches in fishery science and its related fields.

Laboratory of Food Chemistry

1949

1)* W. SIMIDU, Studies on muscle of marine animals. VI. Distribution of extractive nitrogens in the muscle of tuna, *Thunnus orientalis*. Bull. Japan. Soc. Sci. Fish., **15**, 29–31.

2)* W. SIMIDU, Studies on muscle of marine animals. VII. Analysis of various fish meats. Bull. Japan. Soc. Sci. Fish., **15**, 32–34.

3)* W. SIMIDU, Studies on muscle of marine animals. VIII. Distribution of exractive nitrogen on various fishes. Bull. Japan. Soc. Sci Fish., **15**, 35–40.

4)* W. SIMIDU, Kamaboco. Seikatsusha, Tokyo, pp. 1–288.

1951

1)* W. SIMIDU, On the fish muscle proteins. Bull. Japan. Soc, Sci. Fish., **16**, 201–202.

2)* W. SIMIDU & H. TAKUMA, Studies on muscle of aquatic animals. IX. Change of the heat coagulation curve of fish muscle plasma. Bull. Japan. Soc. Sci. Fish., **17**, 103–105.

3)* W. SIMIDU & K. ÔISI, Studies on putrefaction of aquatic products. I. The mechanism and the speciality of development of ammonia in the shark muscle. Bull. Japan. Soc. Sci. Fish., **16**, 423–427.

4)* W. SIMIDU & K. ÔISI, Studies on putrefaction of aquatic products.II. Variation of urease in the shark muscle. Bull. Japan. Soc. Sci. Fish., **17**, 38–40.

5)* W. SIMIDU & K. ÔISI, Studies on putrefaction of aquatic products. III. Analysis of urea and urease-activity. Bull. Japan. Soc. Sci. Fish., **17**, 99–102.

6)* W. SIMIDU & K. ÔISI, Studies on putrefaction of aquatic products. IV. Observation on speciality for development of ammonia in shark muscles (1). Bull. Japan. Soc. Sci. Fish., **16**, 29–30.

7)* W. SIMIDU & K. ÔISI, Studies on putrefaction of aquatic products. V. On urea in elasmobranchiate fishes. Bull. Japan. Soc. Sci. Fish., **16**, 31–33.

8)* W. SIMIDU & S. UENO, Studies on Electrical Sterilization. I. Non-septum electrolysis of sodium chloride solution with direct current. Bull. Japan. Soc. Sci. Fish., **16** 34–36.

9)* W. SIMIDU & T. YAMADA, Studies on fish-oil. I. Extraction of liver-oil from the migrating fishes. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **5**, 29–33.

10)* W. SIMIDU & Y. TOYOHARA, Studies on fish-oil. II. Experiments on deodorization of fish oil, especially on polymerization by heat. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **6**, 19–22.

1952

1)* W. SIMIDU & K. TAKEDA, Studies on muscle of aquatic animals. X. Hydration and dehydration of fish muscle. Bull. Japan. Soc. Sci. Fish., **17**, 132–134.

2)* W. SIMIDU & S. HIBIKI, Studies on muscle of aquatic animals. XI. On the denaturation of fish muscle protein caused by freezing and salting, and the relation between them. Bull. Japan. Soc. Sci. Fish., **17**, 301–304.

3)* W. SIMIDU & M. TAKEDA, Studies on muscle of aqutic animals. XII. Distribution of extractive nitrogens in muscle of squids. Bull. Japan. Soc. Sci. Fish., **18**, 233-237.

4) W. SIMIDU & K. ÔISI, Biochemical Studies on the urea in the muscle of elasmobranchiate fish. Memoirs Coll. Agr., Kyoto Univ., No. **62**, 45–62.

5)* W. SIMIDU, Distribution of extractive nitrogens in several kinds of dried cuttlefish, with special reference to their taste. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **9**, 1–7.

6)* W. SIMIDU & K. TAKEDA, Studies on making fish soy from fish offals. A Special Publication of the Japan Sea Regional Fisheries Reserch Laboratory on the 3rd Anniversary of its Foundation, 259–262.

1953

1)* Y. SHIMIZU & W. SIMIDU, Studies on muscle of aquatic animals. XIII. On solubility of coagulable protein in neutral salt solutions. Bull. Japan. Soc. Sci. Fish., **19**, 753–756.

2)* Y. SHIMIZU & W. SIMIDU, Studies on muscle of aquatic animals. XIV. Effect of neutral salts on protein denaturation. Bull. Japan. Soc. Sci. Fish., **19**, 757-760.

3)* W. SIMIDU, T. HIGASHI & T. ISIKAWA, Studies on muscle of aquatic animals, XV. Imidazol compounds in fish muscles, with special reference to the taste of red muscle fishes. Bull. Res. Inst. Food Sci., Kyoto Univ., No.**10**, 78–82.

4)* W. SIMIDU, S. HIBIKI, S. SIBATA & K. TAKEDA, Studies on muscle of aquatic animals XVI. Distribution of extractive nitrogens in muscles of several kinds of gastropod, Bull. Japan. Soc. Sci. Fish., **19**, 871–875.

5)* W. SIMIDU, Y. KUROKAWA & S. IKEDA, Studies on muscle of aquatic animals. XVII. Imidazol compounds in fish muscles, with special references to the taste of red muscle fishes. (2). Bull. Res. Inst. Food Sci., Kyoto Univ., No. **12**, 40–48.

6)* W. SIMIDU, S. IKEDA & Y. KUROKAWA, Studies on muscle of aquatic animals. XVIII. On L-histidine decarboxylase. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **12**, 49–56.

7)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products VI. Observation on

speciality for development of ammonia in shark muscles (2). Bull. Japan. Soc. Sci. Fish., **19**, 19–22.

8)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. VII. On urea-splitting bacteria in shark muscle. Bull. Japan. Soc. Sci. Fish., **19**, 159–161.

9)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. VIII. On putrefaction of squid muscle. Bull. Japan. Soc. Sci. Fish., **19**, 877–881.

10)* W. SIMIDU, S. HIBIKI & M. HUJITA, Studies on putrefaction of aquatic products. IX. Supplement on putrefaction of shark muscle.——On volatile base developing in shark musle. Bull. Japan. Soc. Sci. Fish., **19**, 882–885.

11)* Y. SHIMIZU & W. SIMIDU, Studies on Jelly-strenth of Kamaboko. I. Estimation of jelly-strength of Kamaboko. Bull. Japan. Soc. Sci. Fish., **19**, 596–602.

1954

1)* W. SIMIDU, M. HUJITA & K. ENDO, Studies on muscle of aquatic animals. XIX. Determination of glycine by nitroanilic acid. Bull. Japan. Soc. Sci. Fish., **20**, 305–307.

2)* W. SIMIDU & M. HUJITA, Studies on muscle of aquatic animals. XX. Distribution of extractive nitrogens in mulscle of shrimps, with special reference to their taste. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **14**, 18–24.

3)* W. SIMIDU & M. HUJITA, Studies on muscle of aquatic animals. XXI. On glycine content in extractive of shrimps, with special reference to their taste. Bull. Japan. Soc. Sci. Fish., **20**, 720–722.

4)* K. ENDO, M. HUJITA & W. SIMIDU, Studies on musle of aquatic animals. XXII. On distribution of extractive nitrogens and free glycine content in squids. Bull. Japan. Soc. Sci. Fish., **20**, 723–725.

5)* W. SIMIDU & I. TIHARA, Studies on putrefaction of aquatic products. X. On putrefaction of salted and dried fishes. Bull. Japan. Soc. Sci. Fish., **20**, 30–32.

6)* W. SIMIDU & T. KIRIYAMA, Studies on putrefaction of aquatic products. XI. Effect of antiseptics on fish muslces. Bull. Japan. Soc. Sci. Fish., **20**, 33–35,

7)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XII. On putrefaction of bloody muscle. Bull. Japan. Soc. Sci. Fish., **20**, 206–208.

8)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XIII. Comparison on putrefaction of different kinds of fish (1). Bull. Japan. Soc. Sci. Fish., **20**. 298–301.

9)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XIV. Comparison on putrefaction of different kinds of fish (2). Bull. Japan. Soc. Sci. Fich., **20**, 302–304.

10)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XV. Comparison of putrefaction for round, fillet, minced and denatured fishes. Bull. Japan. Soc. Sci. Fish., **20**, 388–391.

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12)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XVII. Consideration of difference in putrefaction for various kinds of fish (2). Bull. Japan. Soc. Sci. Fish., **20**, 717–719.

13)* W. SIMIDU & S. NIKAIDO, Studies on electrical Sterilization. II. Electrolysis of sodium chloride solution through alternating current. Bull. Japan. Soc. Sci. Fish., 19, 1154–1156.

14)* W. SIMIDU, S. UENO & T. HARADA, Studies on electrical sterilization. III. Bactericidal

effect of some disinfectants in czapek bouillon. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **12**, 57–65.

15)* W. SIMIDU, S. UENO & T. HARADA, Studies on electrical sterilization. IV. Thermal bactericidal effect of certain disinfectants on non-spore bacteria. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **12**, 66–72.

16)* W. SIMIDU & S. UENO, Studies on electrical sterilization. V. Thermal bactericidal effect of certain disinfectants on spores of *Bacillus mesentericus*. Bull. Res. Inst. Food Sci. Kyoto Univ., No. **14**, 1–5.

17)* W. SIMIDU & S. UENO, Studies on electrical sterilization. VI. Thermal bactericidal effect of certain disinfectants on spores of *Bacillus mycoides*. Bull. Res. Inst. Food Sci. Kyoto Univ., No. **14**, 6–9.

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20)* Y. SHIMIZU, W. SIMIDU & T. IKEUCHI, Studies on jelly-strength of kamaboko. II. Influence of pH on jelly-strength. Bull. Japan. Soc. Sci. Fish., **20**, 209–212.

21)* Y. SHIMIZU, W. SIMIDU & T. IKEUCHI, Studies on jelly-strength of kamaboko. III. Comparison of jelly-strength judged by senses and measured by gelometer. Bull. Japan. Soc. Sci. Fish., **20**, 292–294.

22)* Y. SHIMIZU, W. SIMIDU, & T. IKEUCHI, Studies on jelly-strength of kamaboko. IV. Influence of salts (1). Bull. Japan. Soc. Sci. Fish., **20**, 295–297.

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1)* K. ENDO & W. SIMIDU, Studies on muscle of aquatic animals. XXIII. Distribution of extractive nitrogens in bloody muscle. Bull. Japan. Soc. Sci. Fish., **21**, 127–129.

2)* W. SIMIDU, S. HIBIKI, & S. NAGASAKI, Studies on putrefaction of aquatic products. XVIII. On putrefaction of some white-muscle fishes, mollusca and shrimp. Bull. Japan. Soc. Sci. Fish., **20**, 804–807.

3)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XIX. Influence of certain substances upon histamine formation. Bull. Japan. Soc. Sci. Fish., **20**, 808–810.

4)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XX. Consideration of difference in putrefaction for various kinds of fish (3)—Influence of autolysis on putrefaction. Bull. Japan. Soc. Sci. Fish., **21**, 267–270.

5)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XXI. Consideration on difference in putrefaction for various kinds of fish (4)—On difference of concentration of histidine between the interior and the exterior of cells. Bull. Japan. Soc. Sci. Fish., **21**, 357–360.

6)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XXII. On poisoning of spoiled fish. Bull. Japan. Soc. Sci. Fish., **21**, 361–364.

7)* W. SIMIDU & S. HIBIKI, Studies on putrefaction of aquatic products. XXIII. On the critical concentration of poisoning for histamine. Bull. Japan. Soc. Sci. Fish., **21**, 365–367.

8)* W. SIMIDU & S. UENO, Studies on electrical sterilization. IX. Thermal bactericidal effect of certain disinfectants by two steps gradual heat sterilization. Bull. Japan. Soc.

Sci. Fish. **20**, 927–929.

9)* W. SIMIDU & S. UENO, Studies on electrical sterilization. X. Influence of concentration of disinfectants upon thermal bactericidal effect for spores of *Bacillus mesentericus*. Bull. Japan. Soc. Sci. Fish., **20**, 930–933.

10)* W. SIMISU & S.UENO, Studies on electrical sterilization. XI. Influence of concentration of disinfectants upon thermal bactericidal effect for spores of *Bacillus mycoides*. Bull. Japan. Soc. Sci. Fish., **20**, 934–936.

11)* Y. SHIMIZU, T. IKEUTI & W. SIMIDU, Studies on jelly-strengh of kamaboko. V. Influence of freshness of material fish. Bull. Japan. Soc. Sci. Fish., **20**, 811–813.

12)* Y. SHIMIZU, & W. SIMIDU, Studies on jelly-strength of kamaboko. VI. On jelly-strength of starch pastes. Bull. Japan. Soc. Sci Fish., **20**, 893–894.

13)* Y. SHIMIZU & W. SIMIDU, Studies on jelly-strength of Kamaboko. VII. Improvement of jelly-strength of kamaboko with starch (1). Bull. Japan. Soc. Sci. Fish., **20**, 895–897.

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15)* Y. SHIMIZU & W. SIMIDU, Studies on Jelly-strength of Kamaboko. IX. On influence of salts (2). Sodium chloride. Bull. Japan. Soc. Sci. Fish., **21**, 501–502.

16)* T. IKEUTI & W. SIMIDU, Studies on protein-oil-water system in kamaboko. I. Effect of added oil on jelly-strength of kamaboko. Bull. Japan. Soc. Sci. Fish., **20**, 814–815.

17)* W. SIMIDU, S. HIBIKI & A. NAKAGOSI, Studies on utilization of fish-wastes. I. On preservation with cereals, their offals and saw-dust(1). Bull. Japan. Soc. Sci. Fish., **21**, 130–133.

18)* W. SIMIDU, S. HIBIKI & A. NAKAGOSI, Studies on utilization of fish-wastes. II. On preservation with cereals, their offals and saw-dust (2). Bull. Japan. Soc. Sci. Fish., **21**, 134–137.

1956

1)* W. SIMIDU & K. ENDO, Studies of muscle of aquatic animals. XXIV. Determination of betaine nitrogen. Bull. Japan. Soc. Sci. Fish., **22**, 413–416.

2) W. SIMIDU & S. UENO, Studies on electrical sterilization. XII. Bacteriostatic effect of disinfectants on heated spores (1). Memoirs Res. Inst. Food Sci., Kyoto Univ., No. **20**, 25–29.

3) W. SIMIDU & S. UENO, Studies on electrical sterilization. XIII. Bacteriostatic effect of disinfectants on heated spores (2). Memoirs Res. Inst. Food Sci., Kyoto Univ., No. **10**, 30–33.

4)* W. SIMIDU, Fish-sausages. Zenkoku Gyoniku-sōsēji Kyokai, Tokyo. pp. 1–198.

1957

1)* W. SIMIDU, S. UENO & M. HUJITA, Studies on fish sausage. I. On properties of casing. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **19**, 35–43.

2)* W. SIMIDU, S. UENO, S. HIBIKI, M. HUJITA, Y. SHIMIZU, K. ENDO, U. SIMIZU, T. IKEUTI, I. TAKAGI, H. TERASIMA & A. NAKAGOSI, Studies on fish sausage. II. Chemical compositions and effects of storage. Bull. Res. Inst. Food Sci., Kyoto Univ., No. **19**, 44–51.

3) W. SIMIDU & S. HIBIKI, Studies on Putrefaction of aquatic products. XXIV. On spoilage

of some shell-fishes. Bull. Japan. Soc. Sci. Fish., **23**, 255-259.

Physical Chemistry Laboratory

1949

1)* T. KAWAKAMI & Y. IIJIMA, Estimation of the freezing time for fishes in terms of Plank- Langthtroth's equation. Refrigeration, No. **261**, 1-6.

1951

1) H. FUJITA, On the problem of heat conduction at high temperatures. Memoirs Coll. Agr., Kyoto Univ., No. **59**, 31-42.

2) H. FUJITA, On the approximate solution of one-dimensional freezing equation. I. Memoirs Coll. Agr., Kyoto Univ., No. **59**, 43-53.

1952

1)* H. FUJITA, On the approximate solution of one- dimensional freezing equation. II. Bull. Japan. Soc. Sci. Fish.,**17**, 197-205.

2)* H. FUJITA, A certain non-linear problem of thermal conduction in a can. Bull. Japan. Soc. Sci. Fish., **17**, 393-400.

3) H. FUJITA, A numerical solution of the differential equation for adsorption-controlled diffusion in a solid. Textile Research J., **22**,281-286.

4) H. FUJITA, On the distribution of liquid ascending in a filter paper. J. Phys. Chem., **56**, 625-629.

5) H. FUJITA, A theory of percolation for the case of two solutes. J. Phys. Chem., **56**, 949-953.

6) H. FUJITA & A. KISHIMOTO, A method of determining the concentration dependence of the diffusion coefficient. Textile Research J., **22**, 94-95.

7) H. FUJITA, The exact pattern of a concentration-dependent diffusion in a semi-infinite medium. I. Textile Reserch J., **22**, 757-760.

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9) H. FUJITA, K. NINOMIYA & T. HOMMA, Mechanical properties of concentrated hydogels of agar-agar. I. Modulus of elasticity in compression. Bull. Chem. Soc. Japan, **25**, 374-378.

10) T. KAWAKAMI, Drying and spoilage of fish. Memoirs Coll. Agr., Kyoto Univ., No. **62**, 37-43.

1953

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2) H. FUJITA & K. NINOMIMA, Mechanical properties of concentrated hydrogels of agar-agar. III. Relaxation of stress in compression. Bull. Chem. Soc. Japan, **26**, 24-29.

3) H. FUJITA & A. KISHIMOTO, Further Studies on the method of determinig the concentration dependence of the diffusion coefficient. Textile Research J., **23**, 59-63.

4)* H. FUJITA & A. KISHIMOTO, On the relaxation of stress in cotton plied yarns. J. Soc. Cellulose & Textile Ind. Japan, **9**, 74–78.

5)* H. FUJITA, Approximate methods for determining relaxation time distributions from experimental stress relaxation data. Busseiron-Kenkyu, No. **63**, 48–64.

6) H. FUJITA, Diffusion with a sharp moving boundary. J. Chem. Phys., **21**, 700–705.

7)* H. FUJITA & A. KISHIMOTO, Diffusion accompanied with adsorption, with special reference to the dyeing of fibers. J. Soc. Cellulose & Textile Ind. Japan, **8**, 244–249.

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9)* A KISHIMOTO, The relaxation of stress in drawn Amilan. J. Soc. Cellulose & Textile Ind. Japan, **8**, 621–625.

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Anatomy and Relationships of the Japanese Fishes of the Family Gempylidae*,**

By

Kiyomatsu MATSUBARA and Tamotsu IWAI***

With 3 Tables and 14 Text-figures

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I. Introduction

The Japanese group of the Gempylidae, a family of the suborder Scombrina, comprises 9 genera and 9 species. Each species, with exception of *Mimasea taeniosoma* KAMOHARA which is known only from the Japanese area, appears to occur, at least seasonally, throughout all warm waters of the world. Almost all species are presumably inhabiting the deep-sea bathypelagic and archibenthic zone at depths varying from 100 to 300 meters and are occasionally captured by deep-sea moter trawlers or tuna long lines (e.g. JOHNSON et al., 1956).

An extensive literature, involving our previous report (MATSUBARA and IWAI, 1952)

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*Although WHITLEY (1951) advocated Acinaceidae as a scientific name of this family, BERG (1940) and TUCKER (1955) advised that Gempylidae should be used as the family name. We follow herein the TUCKER's opinion.

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***Zoological Laboratory

has accumulated, dealing with the taxonomy of the fishes of this group based upon the external features, and the distinguishable characteristics of each species have convincingly demonstrated. However, the mutual relationships of these fishes still are far from being satisfactorily understood. The definite conclusions to this problem, as is generally believed, could be found only in accurate and adequate comparative studies based on the anatomical features of all species referred to this family. As to the internal features of the gempylid fishes, little speculation has hitherto been done, though STARKS (1911) worked on the osteology of *Promethichthys prometheus* (CUVIER) and though KISHINOUYE (1926) reported the same of *Lepidocybium flavobrunneum* (SMITH). That such works have to date been unsuccessful in providing an indisputable understanding of the phylogenetic status of this group, would necessitate acquisition of much more detailed knowledge on the comparative anatomy. Hence the present investigation, although supplemental, was undertaken in order to ascertain the interrelationships among the genera of this family represented from Japan, especially standing on the viewpoints of osteology and visceral anatomy.

Incidental to the chief aim stated above, a secondary object of this paper is to determine the taxonomic status of the genus *Neoepinnula* which was proposed by us in the previous publication (MATSUBARA and IWAI, 1952), for *Neoepinnula* seems to be neglected by a recent ichthyologist (GREY, 1953).

MUNRO (1949) presented a comprehensive revision of *Lepidocybium flavo-brunneum* (SMITH), and added this species to the family Gempylidae based chiefly upon the characters of caudal skeleton. He did not treat of the other skeletal system or visceral anatomy. In this work, a careful examination has done on these characteristics, and an attempt has made to clarify the relationship of *Lepidocybium* to the other gempylid fishes.

It was impossible to examine the skeletal system of *Nealotus tripes* JOHNSON, a member of this family, because only a single specimen was available to us. Therefore, the discussion of the phylogenetic status of this species should be omitted in this paper.

II. Materials and Methods

The observations to be described were performed with eight species of the family Gempylidae obtained from deep waters off Owashi, Mie Prefecture and off Kôchi, Kôchi Prefecture. A detailed account of the localities and measurments of each species is given in Table I.

In making the osteological preparations, excellent results were obtained by using CLOTHIER's modification (1950) of HOLLISTER's technique for the clearing and staining.

III. Comparative Anatomy

1. Lateral line and scales

Description : All species of the family Gempylidae are provided with the distinct tubular lateral line running just beneath the skin. The lateral line is armed with

slender tubular scales which are arranged end to end, forming a continuous chain along the line (Fig. 1, A-H). There is no substantial difference in the structure of the lateral line of each species, but conspicuous specific differences are to be determined in its location so that it may serve one of the specific characteristics. These might be roughly classified into four major groups on the basis of the location, and each is characterized as follows : (1) The lateral line is greatly undulated, (2) It is merely a single line, (3) It is bifurcated ; the lower branch running along the lower contour of the body, and (4) It is bifurcated as is the case with the preceding type, but the lower branch runs along the middle line of the body.

Table 1. Localities and measurements of 8 species belonging to the family Gempylidae

Species	Number of dissected specimens	Standard length	Date of collection	Locality
Lepidocybium flavo-brunneum (SMITH)	1	395.5mm.*	Nov. 3, 1954	Owashi
Ruvettus pretiosus COCCO	1	270.5*	Nov. 12, 1952	Owashi
Neoepinnula orientalis (GILCHRIST and von BONDE)	2	182.0, 191.0*	April 10, 1954	Kôchi
Epinnula magistralis POEY	1	215.0*	Nov. 12, 1952	Owashi
Rexea solandri (CUVIER)	5	125.0–215.5*	April 10, 1954	Kôchi
Promethichthys prometheus (CUVIER)	2	245.0, 267.0*	Nov. 12, 1952	Owashi
Mimasea taeniosoma KAMOHARA	1	390.5*	April 10, 1954	Kôchi
Gempylus serpens CUVIER	1	488.0*	June, 1952	Owashi

The first type of the lateral line is assigned to *Lepidocybium.*** In this species, it begins above the upper angle of gill-opening and runs backward along the dorsal margin of the trunk to below the base of fourth dorsal spine where it dips abruptly. Then it drops down straight to the ventral border of the trunk between the pelvic fin and anus, where it turns dorso-posteriorly. Thence it reaches to midline of the body above the origin of anal fin. There it turns again ventro-posteriorly. After drawing a loose arc, it finally continues to the keel on the caudal peduncle. The lateral line scales are embedded under the tough integument armed with unique scale networks (Fig. 1A).

The second type is represented by two species, *Ruvettus* and *Promethichthys.* Both species are marked by having a single lateral line, but some differences between these two species can be observed in detailed structure. In *Ruvettus,* the lateral line arises above the gill-opening and runs nearly straight all the way to base of the caudal fin. The tubular scales of the lateral line are covered with peculiar tridentate body scales having a forked sharp spine at each posterior extremity (Fig. 1 B). Therefore,

* All drawings of skeletons and visceral organs were made on these specimens.

**SCHULTZ and SPRINGER (1956) failed to find any trace of a lateral line in a large specimen of this species measured 1,210 mm. fork length, and 1,290 mm. total length.

the detail of the lateral line is indiscernible without staining. Now, in *Promethichthys* the lateral line descends rather sharply downward and backward on the anterior part of the body. Then it extends straight backward far beyond the base of the middle caudal ray. The scale of the lateral line is a rather slender tubule (Fig. 1 E).

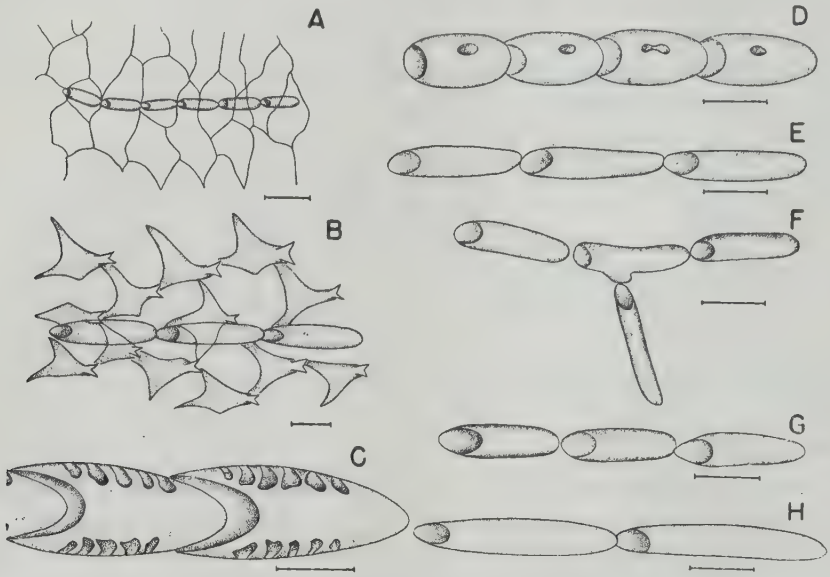


Fig. 1. Scales of anterior portion of left lateral line in 8 species of the Japanese gempylids. A, *Lepidocybium flavo-brunneum ;* B, *Ruvettus pretiosus ;* C, *Neoepinnula orientalis ; D, Epinnula magistralis ;* E, *Promethichthys prometheus ;* F, *Rexea solandri ;* G, *Mimasea taeniosoma ;* H, *Gempylus serpens*. Each scale indicates 1 mm.

Epinnula and *Neoepinnula* belong to the third type. In the fish belonging to the former genus, the lateral line begins above the angle of gill-opening and runs backward to the point of bifurcation below fifth or sixth dorsal spine. The upper branch courses directly backward along the dorsal contour of the body to the base of caudal fin. The lower branch descends directly downward to behind the base of pectoral fin, where it curves ventro-posteriorly to above the origin of ventral fin. Finally it extends backward along the ventral contour of the body to the base of caudal fin. Consequently, the posterior end of either branch confronts each other at the caudal peduncle. The scale in the lateral line is keg-like in shape and is perforated by a small ovoid pore near the center (Fig. 1 D). In *Neoepinnula,* both upper and lower lateral lines originate on the upper angle of gill-opening. The upper lateral line runs backward along the dorsal contour of the body and ends below dorso-anterior end of the caudal fin. The lower one descends downward and slightly backward to below the lower edge of the base of pectoral fin, then it runs ventro-anteriorly to the ventral border of the trunk below the anterior end of the base of the pectoral fin. Finally it extends backward along the ventral contour of the body to the base of the middle caudal ray. Each

lateral line scale is provided with 5–7 irregular hollows along both the dorsal and ventral margin (Fig. 1 C). It is impossible to determine whether these fenestellae are distinct specific characteristics or whether these are formed by chance due to inadequate calcification of the scale. At any rate, however, it is peculiar that all lateral line scales of this species possess such fenestellae.

The last type contains three genera, *Rexea*, *Mimasea* and *Gempylus*. In former two genera, the lateral line originates on the upper angle of gill-opening and runs backward to the point of the bifurcation which is seen below the membrane between fourth and fifth dorsal spines. Then, the upper branch extends backward along the dorsal contour of the body to below middle of the base of soft dorsal fin in *Rexea*, and to below the penultimate dorsal spine in *Mimasea* respectively. From the bifurcation, the lower branch in the fish of both genera drops sharply to midline of the body and then it runs straight to the caudal base. Sometimes, however, the lower branch of *Rexea* irregularly undulated on the posterior part of the body. In *Gempylus*, two lateral lines begin simultaneously above the upper angle of opercle. The upper one runs backward near the dorsal border of the body to below the base of the last dorsal spine. The lower line descends gently ventro-posteriorly to near the tip of pectoral fin, and then, extends along midline of the body to the caudal penduncle. In these three genera, no remarkable diversity is observed in the structure of tubular lateral line scales (Fig. 1 F–H).

Discussion : The lateral line of *Lepidocybium* is conspicuously unique. It is uncommon in the teleostean fishes to have such greatly undulated lateral line. However, attention should be called to the lateral line of fishes of the genus *Scomberomorus* which are considered to be closely related members of the gempylid fishes. In certain species of the genus *Scomberomorus*, for instance *S. commerson*, the lateral line deeply inflects below the first five dorsal finlets as is the case with *Lepidocybium*. The lateral lines of these two species are similar with each other in shape, except for the anterior half of the line. It is therefore conceivable that *Lepidocybium*-type lateral line is specialized from *Scomberomorus*-type.

Although for convenience sake we classified the lateral line of both *Ruvettus* and *Promethichthys* in the same group, there is a substantial difference between these two lateral lines. The lateral line of *Ruvettus* is almost straight and is a normal form of teleostean fishes. In *Promethichthys*, it is also a single line, but it drops down suddenly at anterior part, and looks like the double lateral lines that disappeared only the upper branch out of our sight. Accordingly, we may be able to surmise that this is a modified type of the double lateral lines of fourth group caused by reduction of the upper branch.

That a majority of the gempylid fishes have highly specialized double lateral lines is of sufficient magnitude to consideration of its origin. In regard to the origin of the upper branch of the double lateral lines, two conjectures could be offered from a viewpoint of the nervous supply. According to RAY (1950), most of the fishes possess a ramus dorsalis which arises from the stem of the nervus lineae lateralis and runs, just under the skin, posteriorly along the dorso-lateral contour of the body. This route

coincides with that of the upper branch of the bifurcated lateral line. One hypothesis is that the upper branch was developed by nervous supply of a ramus dorsalis. On the other hand, ALLIS (1903) reported that, in *Scomber*, the nervus lineae lateralis occurs as two parallel nerves. One branch named nervus lineae lateralis superficialis lies just beneath the skin and the other one named nervus lineae lateralis profundus is more deeply embedded. In this case, it is also conceivable that the upper branch of lateral line is led from the transmigrated nervus lineae lateralis profundus lying just beneath the skin of the dorso-lateral contour of the body. In comparison of third type of double lateral lines with fourth one, the latter one shows more generalized appearance than the former one, because the lower branch of the latter runs along the midline of the body that is general pattern of the route of the lateral line in most of the teleostean fishes. Obviously, the third type is specialized from the fourth type. It is interesting to note that the lateral line of *Grammatorcynus bicarinatus* (QUOY and GAIMARD), a member of the family Scombridae, resembles that of *Epinnula*. *Grammatorcynus* shows close affinity with the gempylid group not only in feature of the lateral line but also in the vertebral counts (31 in number).

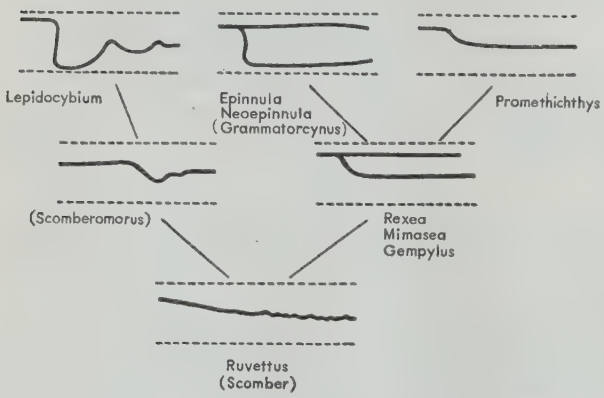


Fig. 2. Mutual relationships of various types of the lateral line of the Japanese gempylid fishes. Generic names parenthesized are of fishes of the family Scombridae.

Thus, mutual relationships of each type of the lateral line of gempylid fishes are summarized in Fig. 2.

Certainly, as aforementioned, the gempylid fishes are extremely variable in the lateral line characteristic. One cannot be sure that these genera providing such scattered characteristics represent a single family. GREY (1953) was puzzled over this problem as well as some other features. However, in some other teleostean families, more variant lateral lines are recognizable. For example, in the family Hexagrammidae, fishes of the genus *Pleurogrammus* have 4 lateral lines and those of the genus *Hexagrammos* have 4 or 5 lateral lines, whereas *Agrammus* has only a single lateral line.

2. Jaws

Description : In all species examined, with exception of *Gempylus*, the upper jaw consists of well developed premaxillary, maxillary and supramaxillary (Fig. 3 A-G). In *Gempylus*, however, the skeletal elements constituting the upper jaw are tightly bound together and the border of each element is obscure (Fig. 3 H). The premaxillary forms both the tip of the snout and the whole upper margin of the mouth. Anteriorly it curves proximally to form a symphysis with its counterpart of the opposite side.

At the anterior end it projects dorso-posteriorly to form the pedicel. The premaxillary pedicel is nearly vertical in *Epinnula*, *Neoepinnula* and *Rexea*, slightly inclined backward in *Lepidocybium*, *Ruvettus* and *Promethichthys*, remarkabley inclined backward in *Mimasea*, and nearly horizontal in *Gempylus*. In *Mimasea*, and especially in *Gempylus*, the ventro-anterior extremity of the premaxillary is rather sharply pointed. This, as well as the inclination of the pedicel, may contribute to the acutely pointed snout in both species (Fig. 3 G, H). Posteriorly, the premaxillary tapers and enters the angle of the gape where it is connected with the postero-proximal side of the maxillary by a ligament. Usually, the premaxillary bears one or more strong, compressed canine-like teeth anteriorly except for *Lepidocybium* in which the bone is armed uniformly with small teeth. Some of canine-like teeth have ligamentous attachments which permit them to be depressed only backward. External to these, a row of small but strong teeth is arranged. A large slender maxillary lies on the dorso-posterior edge of the premaxillary. It has a prominent knob at the anterior end by which it articulates with the cranium. Posteriorly, it gradually expands and forms the posterior corner of the upper jaw.

The splint-like supramaxillary rests on the dorso-posterior edge of the maxillary. It is nearly or slightly more than half as long as the maxillary. In *Lepidocybium*, the bone is much shorter than the maxillary and rudimental when viewed from the outside of the bone.

The lower jaw is made up of the dentary, articular and angular. The dentary is the largest bone in the mandible and forms the anterior tip of this apparatus. Anteriorly it meets the counterpart of the opposite side at the symphysis. Posteriorly it deeply forked, between which the anterior part of the articular is inserted. The posterior part of the dentary is greatly expanded in *Lepidocybium*, *Epinnula* and *Neoepinnula*, but it is rather slender in *Ruvettus*, *Rexea* and *Promethichthys*. It is extremely slender in *Mimasea* and *Gempylus*. A single row of strong, moderately large teeth is set on the upper margin of the dentary. The articular is also large and is joined with the dentary anteriorly by the overlapping processes of the latter. Posteriorly it tapers and articulates with the quadrate. The angular is small and rests on the posterior

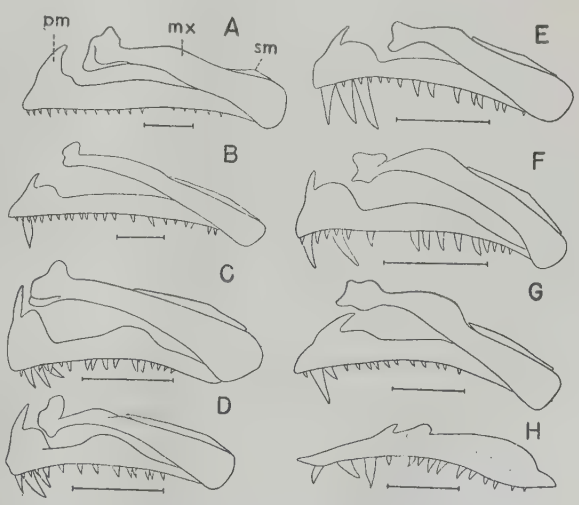


Fig. 3. Upper jaw of 8 species of the Japanese gempylids. A, *Lepidocybium flavo-brunneum*; B. *Ruvettus pretiosus*; C, *Neoepinnula orientalis*; D, *Epinnula magistralis*; D, *Promethichthys prometheus* F, *Rexea solandri*; G, *Mimasea taeniosoma*; H, *Gempylus serpens*. mx, maxillary; pm, premaxillary; sm, supramaxillary. Each scale indicates 10 mm.

angle of the articular. It forms the posterior edge of the mandible.

Discussion : In *Gempylus* the skeletal elements of the upper jaw show an unusual structure as compared with those of the other species of this family. Because each element is firmly ankylosed the structural detail is not clearly detected. But it seems likely that the upper jaw is composed of three elements, the premaxillary, maxillary, and supramaxillary as is the case with the other species. The fact that *Gempylus* possesses a greatly enlarged mouth and stout teeth, suggests that this species is a voracious feeder. Nevertheless the modified upper jaw loses the protractility and, consequently, movement of the upper jaw is strictly limited. This fact alone confronts with the other features suggesting the adaptation to the voracious nature. This unusually modified structure may not be an abnormality. Regrettably, however, an accurate decision is not forthcoming, inasmuch as we had only one specimen to dissect.

MATSUBARA and IWAI (1952) stated as one of important features of *Mimasea* and *Gempylus* that the snout is projecting far beyond the anterior extremity of the premaxillary. The premaxillary of both species, especially of *Gempylus*, is also sharply pointed anteriorly instead of being rather truncated in the other species. It is evident that in these two species, the premaxillary contributes to form the projecting beak-like snout.

It must be pointed out that only *Lepidocybium* lacks the enlarged canine-like tooth on both upper and lower jaws, while other species bear one or more well developed canine-like ones on the tip of jaws. REGAN (1909) placed the canine-like teeth as characteristic of Trichiuriforms represented by Gempylidae and Trichiuridae. TUCKER (1956), in his comprehensive revision of the family Trichiuridae, described that the trichiurid fishes are also provided with sharp canine-like teeth on jaws. It is apparent that the gempylids are more closely related to the trichiurids than to the other scombroid fishes so far as this characteristic is concerned. In this view one may probably entertain doubt whether to assign *Lepidocybium* to the member of the family Gempylidae.

3. Suspensorium and opercular apparatus

Description : There is no fundamental difference among the members of this family in the general arrangement of the skeletal elements of suspensorium and opercular series.

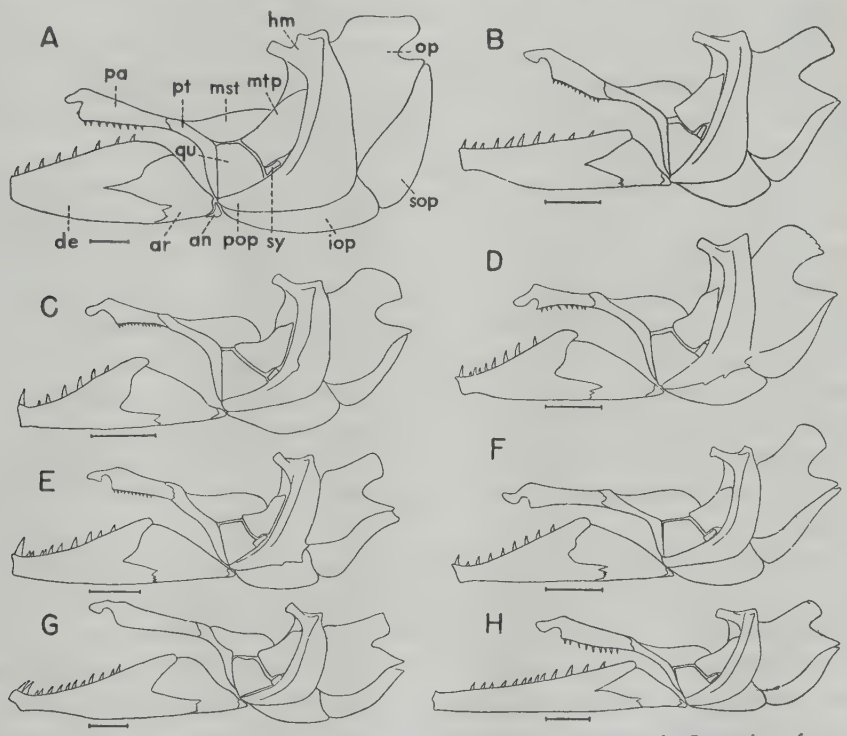
The hyomandibular, roughly wedge-like in shape, is the key suspensorium of the facial bones. Its upper margin has two condyles articulating with the sphenotic anteriorly and with the pterotic posteriorly. The bone is so sturdily joined posteriorly with the preopercle that the boundary with the latter is in fact obscure. The ventral part is greatly overlapped by the dorso-posterior corner of the metapterygoid, and this portion is not seen from the outside. The ventral projection extends downward along the proximal border of the metapterygoid, and rests against the posterior end of the symplectic (hm in Fig. 4).

Below the metapterygoid and above the dorso-posterior border of the quadrate, there is a small but stout rod-like symplectic. The lower extremity of this bone is

slightly inserted into small notch of the dorso-posterior corner of the quadrate (sy in Fig. 4).

The quadrate is a flat bone, fan-like in shape. At the lower edge it bears a condyle, articulating with the posterior end of the articular. Dorsally, it articulates with the mesopterygoid and metapterygoid through an intervention of the cartilage. The bone is joined anteriorly with the pterygoid and posteriorly with the preopercle and partly with the symplectic (qu in Fig. 4).

Dorso-posterior to the quadrate there is a flat metapterygoid. Posterior part of the bone deeply overlies the hyomandibular, and posterior edge meets the anterior border of the preopercle. Thus, it separates the quadrate from the hyomandibular in outer aspect. The anterior half of the upper edge touches the mesopterygoid and the



Fig, 4. Lateral view of the suspensorium and opercular apparatus in 8 species of the Japanese gempylids. A, *Lepidocybium flavo-brunneum ;* B, *Ruvettus pretiosus ;* C, *Neoepinnula orientalis ;* D, *Epinnula magistralis* ; E, *Promethichthys prometheus;* F, *Rexea solandri* ; G *Mimasea taeniosoma ;* H, *Gempylus serpens*. ar, articular ; an, angular ; de, dentary ; hm, hyomandibular ; iop, interopercle ; mst, mesopterygoid ; mtp, metapterygoid ; pa, palatine ; pop, preopercle ; pt, pterygoid ; op, opercle ; qu, quadrate ; sos, subopercle ; sy, symplectic. Each scale indicates 10 mm.

ventral corner meets the symplectic through an intervention of a cartilaginous patch (mtp in Fig. 4).

The mesopterygoid is a thin expanded plate lying almost horizontally. It forms

the main portion of the floor of the orbit. Posteriorly it extends to the dorso-anterior border of the metapterygoid. Ventrally it meets the quadrate. Anteriorly it extends far forward along the dorsal border of the pterygoid nearly to the base of the palatine (mst in Fig. 4).

The pterygoid is a stout, crooked bar, widest at the middle portion, and tapering to both extremities. Anteriorly, its long axis extends upward and forward and unites with the posterior end of the palatine. Hind edge of the bone articulates with both the quadrate and mesopteryoid. Therefore, it forms a strut between the quadrate and the palatine (pt in Fig. 4).

Anterior to the pterygoid there is a slender palatine, bearing the prominent condyle at the anterior extremity for the articulation of the cartilaginous portion lying under surface of the prefrontal. Posteriorly it is sturdily connected with the pterygoid and partly with the mesopterygoid. In *Lepidocybium*, *Ruvettus*, *Epinnula*, *Neoepinnula*, *Promethichthys*, and *Gempylus*, the palatine bears a single row of small conical teeth on the ventral surface. However, in the other two genera, *Rexea* and *Mimasea* it is evidently edentulous (pa in Fig. 4).

The preopercle is a large bone roughly lunate-like in shape, extending along the posterior margin of the suspensorium. The upper half of the anterior border is sturdily joined with the posterior edge of the hyomandibular. The lower limb of the bone extends ventro-anteriorly to the suspensory angle of the mandible, and its dorso-anterior border meets the metapterygoid, symplectic and quadrate respectively. There is no apparent spine on the posterior edge of the preopercle except for genera *Epinnula* and *Promethichthys* in which the bone is armed with several spinules along the ventro-posterior corner or the lower edge of the bone (Fig. 4D, E). The tubular structure enclosing the lateral sensory canal system runs lengthwisely near the anterior margin of the bone and continues into the mandible. But the opening of the canal is not obvious.

The opercle is a large membrane bone, somewhat squarish in shape, and deeply notched at posterior edge. Anteriorly it underlies the posterior edge of the preopercle. The ventral part of the bone lies over the dorsal edge of the subopercle.

The subopercle, a thin triangular plate, lies below the opercle and forms the posterior margin of the opercular series. Anteriorly it is also covered with posterior edge of the interopercle.

Below the preopercle and anterior to the subopercle, there extends a flat interopercle. The upper part of the bone is deeply overlapped by the preopercle. Its posterior edge overlies the anterior part of the subopercle.

Discussion: The operculo-mandibular series clearly tends to become slenderer in elongate species. In *Lepidocybium*, *Ruvettus*, *Epinnula* and *Neoepinnula*, for example, the apparatus is rather deep as compared with that of elongate species, *Mimasea* and *Gempylus;* the apparatus in the latter two is greatly depressed and slender in general appearance. In addition to this evidence, there occurred in these two genera a marked increase in the length of the mandible, the mandible being longer than the longitudinal length of the opercular apparatus. These structural dif-

ferences are manifestly attributed to the superficial differences of the head length among the species as may be judged from the proportional values, such as: the depth of head is about 1.5 times in the head length in *Lepidocybium;* 2.0 in *Ruvettus;* 1.6 in *Epinnula;* 1.5 in *Neoepinnula;* 2.3 in *Rexea;* 2.5 in *Promethichthys;* 3.0 in *Mimasea;* and 3.3 in *Gempylus.*

In his work on the trichiuroid fishes, TUCKER (1956) described the shape of the subopercular margin as one of the taxonomic features of the subfamilies. According to his description, in Aphanopodinae and Lepidopodinae the lower hind margin of the subopercle is convex, instead of being more or less concave in Trichiurinae. As it is obvious in Fig. 4, in the gempylid fishes except for *Promethichthys,* the lower hind margin of the subopercle is slightly rounded and is similar to the Aphanopodine subopercle in shape. In *Promethichthys,* however, the ventro-posterior margin of the bone is slightly concave as is the case with the Trichiurine subopercle. It is of interest to note that the posterior edge of the opercle is deeply notched in all species examined. This may serve as one of the features distinguishing the family Gempylidae. Although TUCKER (1956), discussing the gempylid-trichiurid relationship, took up the deep notch of the opercle as a striking feature in *Nesiarchus* and *Diplospinus,* this opercular notch is apparently present in all gempylid fishes examined by us.

With exception of some elongate species belonging to such genera as *Mimasea* and *Rexea,* the gempylid members are provided with a number of small teeth on the palatine. TUCKER (1956) is of the opinion that the toothed palatine is an important feature to indicate the phyletic relationship between Gempylidae and Trichiuridae. However, because this feature is rather variable by the species (Fig. 4), it appears likely that the palatine teeth are not so important characteristic in considering the gempylid-trichiurid relationship.

4. Cranium

Description: In general physiognomy the cranium of the gempylid fishes resembles that of scombrids. The skeletal elements constituting the cranium and their general arrangement are exactly the same throughout all the species of this family. However, some interesting differences can be seen in form of certain elements of the cranium. In order to sharply define these differences we made some examinations both qualitatively and quantitatively. The measurements of various parts of the cranium described herein are indicated by following categories: (1) The length of the cranium is measured from the tip of the vomer to rear end of the basioccipital bone. (2) The depth of the cranium is the vertical distance at the level of the posterior end of the orbit. (3) The width of the cranium is measured in the widest part between the sphenotic bones. (4) The preorbital length of the cranium is the length between the tip of the vomer and the anterior edge of the orbital cavity. (5) The interorbital width is measured in the widest part of the frontals above the orbit.

Antorbital portion of the cranium: The mesethmoid is an unpaired thick bone constituting the dorso-anterior corner of the cranium. In dorsal view it is a shield-like bone and bears a pair of mesethmoid processes on its anterior portion of either

side. Posteriorly it projects into the space between anterior ends of the frontals. The mesethmoid is much slenderer especially in the fishes of the genera *Rexea, Mimasea* and *Gempylus* as compared with that of the fishes of the other genera (Fig. 5D, E, F).

The vomer is connected by ankylosis with the ventral side of the mesethmoid and

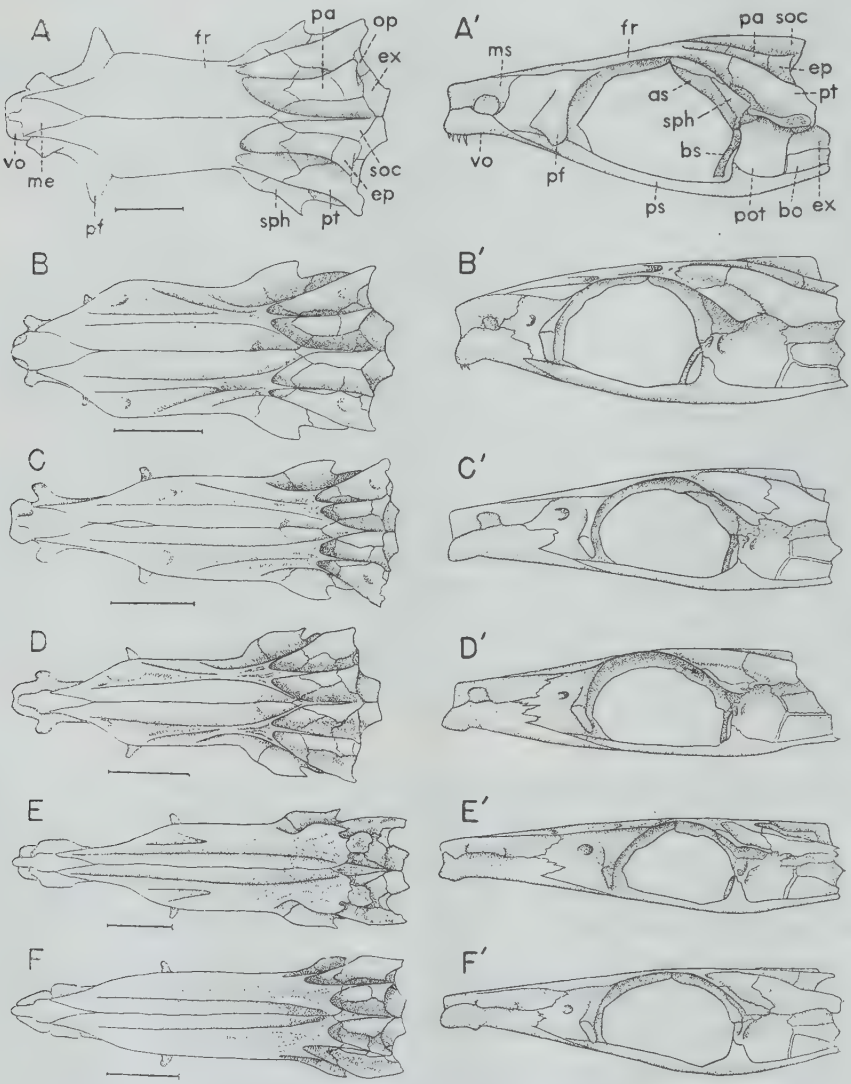


Fig. 5. Dorsal (A–F) and lateral (A′–F′) aspects of the cranium in 6 species of the Japanese gempylids. A·A′, *Lepidocybium flavo-brunneum* ; B·B′, *Ruvettus pretiosus* ; C·C′, *Promethichthys prometheus* ; D·D′, *Rexea solandri* ; E·E′, *Mimasea taeniosoma;* F·F′ *Gempylus serpens*. as, alisphenoid ; bo, basioccipital ; bs, basisphenoid ; ep, epiotic ; ex, exoccipital; fr, frontal ; me, mesethmoid ; op, opisthotic ; pa, parietal ; pas, parasphenoid ; pf, prefrontal ; pot, prootic ; pt, pterotic ; soc, supraoccipital ; sph, sphenotic ; vo, vomer. Each scale indicates 10 mm.

serving as the anterior strut of roof of the mouth. Except for *Lepidocybium* (Fig. 5A), it curves downward at the anterior edge. Posteriorly the ventral surface of this bone tapers gradually and overlies the anterior end of the parasphenoid. In *Lepidocybium*, *Ruvettus* and *Neoepinnula*, the vomer bears several small teeth on its ventro-anterior edge (Fig. 5A′, B′; 6B′). But in the others it is edentulous.

Behind the junction of the vomer and mesethmoid, a pair of prefrontals is present. They meet along the median line and each bears a projection extending ventro-laterally. Posteriorly it draws a boundary to the orbital cavity.

The frontal comprises a pair of large flat bones covering a vast part of the top of the cranium. They are irregularly juxtaposed nearly their whole lengths in the midline, and shortly diverged anteriorly. Anteriorly they reach to the ethmoid region, and posteriorly abut against the supraoccipital, parietals, pterotics and sphenotics. On their ventral surface, above orbit, the frontals hang the alisphenoid. Each frontal bears two sharp longitudinal ridges on posterior surface, one of which continues posteriorly to the parietal ridge and the other to the pterotic ridge. In *Mimasea*, however, the frontals lack these ridges. This bone has two sensory canals, both of which open near surface over the prefrontals. In elongate genera, *Rexea*, *Promethichthys*, *Mimasea* and *Gempylus*, the frontal is rather slender, being about 3 times as long as wide in the former two genera, and further narrower in the others. In the other genera, *Lepidocybium*, *Ruvettus*, *Epinnula* and *Neoepinnula*, the bone is about twice as long as wide.

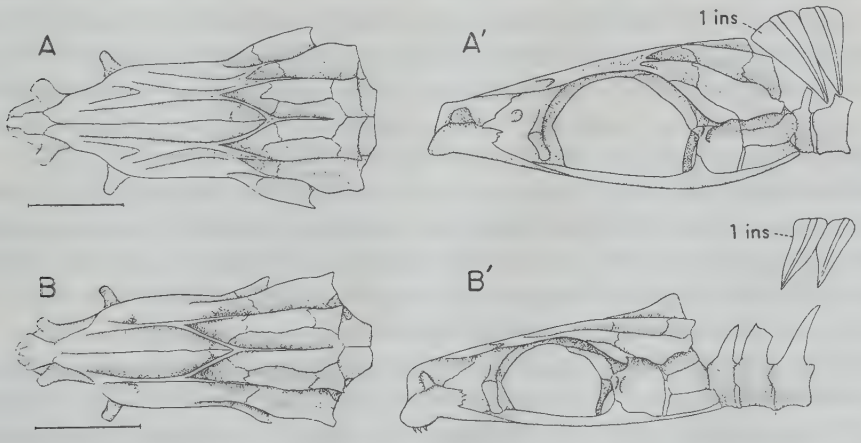


Fig. 6. Dorsal (A·B) and lateral (A′·B′) aspects of the cranium inclusive of lateral aspect of the anteriormost 2 interneural spines and 2 or 3 vertebrae in two species of the Japanese gempylids. A·A′. *Epinnula magistralis*; B·B′. *Neoepinnula orientalis*. 1 ins, first interneural spine. Each scale indicates 10 mm.

The parasphenoid, which is the longest bone running along the midline of the ventral surface of the cranium, is a long narrow bone forming the ventral edge of the cranium. The anterior end of the bone is inserted into the juncture of the mesethmoid

and vomer, and the posterior half is firmly attached to the ventral surface of both the basioccipital and prootics to form the floor of the myodome. It sends a bony wing to ahead of the prootics. The parasphenoid is more or less roundly convex below the basisphenoid in all genera except for *Neoepinnula* in which the bone is nearly straight.

Postorbital portion of the cranium: The supraoccipital is a median bone lying in the upper posterior angle of the cranium and forming a keystone of the posterior portion of the cranium. It meets the frontals anteriorly and separates laterally the epiotics and parietals respectively. The bone is sharply keeled along the midline. Particularly the keel is highly elevated posteriorly in *Neoepinnula*. Posteriorly near the posterior end of the median keel the supraoccipital slopes abruptly downward and articulates with the exoccipital.

The parietals are well-ossified paired bones extending from the posterior edge of frontals to epiotics. These do not fit each other along median line of the cranium, but are separated by an intervention of the supraoccipital. Laterally they meet the pterotics. On the dorsal surface each parietal armed with a longitudinal keel running parallel with the supraoccipital keel. This keel is contiguous to the frontal keel anteriorly and to the epiotic keel posteriorly. In *Mimasea* the dorsal surface of the parietal is flat and unarmed.

The epiotics are a pair of conical bones forming the dorso-posterior corners of the cranium. Each epiotic is bounded anteriorly with the parietal, laterally with the pterotic and mesially with the supraoccipital. Posteriorly it sharply curves downward and connects with the exoccipital and with the opisthotic with exception of *Promethichthys* and *Rexea*. In *Epinnula*, the epiotic meets broadly the counterpart of the opposite side behind the supraoccipital (Fig. 6A). On the dorsal surface of the bone there is a longitudinal sharp crest continuing to the parietal crest. The crest terminates into a pointed tip posteriorly and, consequently, the dorso-posterior edge of the epiotic is sharply pointed. In *Mimasea* the crest is not clear.

The sphenotics are sturdy bones forming the dorso-anterior corners of the brain case. It forms a suture with the frontal and alisphenoid anteriorly, with the prootic ventrally and with the pterotic posteriorly. Laterally each sphenotic is flattened horizontally and forms the lateral frange of the anterior portion of the brain case. The latero-posterior edge of the bone is sharply pointed.

The pterotics, occupying a position behind the sphenotics, end posteriorly in a sharp spike. Particularly, in *Mimasea* and *Gempylus*, the bones project posteriorly and resemble those of *Lepidopus*, a genus of the family Trichiuridae. Each pterotic abuts on the sphenotic and frontal anteriorly, on the parietal and epiotic mesially and on the prootic and opisthotic ventrally. The dorso-lateral border of the bone is highly keeled. This keel is continued to the frontal anteriorly.

The alisphenoids are flat, irregularly shaped bones forming the anterior wall of the brain case and partly the dorso-posterior roof of the orbit. Each alisphenoid articulates with the prootic posteriorly, with the sphenotic dorso-posteriorly and partly with the basisphenoid ventro-posteriorly. Dorsally these are overlaid by the frontals.

Anterior to the median juncture of the prootics, there is a simple, thin rod-like

basisphenoid. It extetends from the upper anterior border of the prootic to the parasphenoid, and articulates with the latter aided by a cartilaginous intervention.

The prootics are rather large bones forming the anterior portion of the lateral wall of the brain case. Each bone participates with the alisphenoid anteriorly and with sphenotic and pterotic dorsally. Ventrally they join along the median line, but the suture is hidden by the parasphenoid. The posterior border of the bone abuts against the opisthotic, exoccipital and basioccipital. A prominent continuous band of the cartilage is intervening between the prootic and the basioccipital or the exoccipital.

The basioccipital is an unpaired median bone, heavily ossified and forming an important portion of the ventro-posterior corner of the cranium. Posterior edge of the bone bears a condyle which makes possible the articulation of the cranium with the anterior edge of the first vertebra. Anteriorly the basioccipital meets the prootic. Dorso-laterally, on each side, it forms the suture with the ventral edge of the exoccipitals. The ventral border is overlain by the parasphenoid.

Exoccipitals form a part of the posterior and lateral portion of the cranium. Posterior to the supraoccipital, the dorso-posterior edge of the bone meets the counterpart of the opposite side at the median line. The lower half of this median suture is pierced by the foramen magnum. Each exoccipital is bordered with the supraoccipital and epiotic dorso-posteriorly, with the opisthotic dorsally, with the prootic anteriorly and with the basioccipital ventrally.

The opisthotics are paired bones curving around the posterior edge of the cranium. The anterior extension of each bone on the ventral surface of the cranium covers the exoccipital-pterotic suture and reaches to the posterior border of the prootic. It also extends to dorsal surface of the cranium and separates the exoccipital from the pterotic for a considerable extent, except for *Promethichthys* and *Rexea* in which these bones are scarcely separated by the opisthotic.

General form of the cranium : A considerable generic difference will be found in the form of the cranium, especially in its depth and width. The depth in length of the cranium is about 2.5 times in *Lepidocybium, Ruvettus* and *Epinnula*, about 3 to 3.5 times in *Neoepinnula, Rexea* and *Promethichthys*, and about 4 times in *Mimasea* and *Gempylus*. The cranium is usually widest at the sphenotic level or pterotic level. The width in length of the cranium is about 2 times in *Lepidocybium, Ruvettus* and *Epinnula*, about 2.5 times in *Neoepinnula* and *Rexea*, about 3 times in *Promethichthys*, and about 3.3 times in *Mimasea* and *Gempylus*. The interobital width in length of the cranium is about 3 times in *Lepidocybium, Ruvettus, Epinnula* and *Neoepinnula*, about 3.5 times in *Promethichthys*, 4 times in *Rexea*, and about 4.5 times in *Mimasea* and *Gempylus*. The preorbital length in length of the cranium is about 3 to 3.5 times in *Lepidocybium, Ruvettus, Epinnula* and *Neoepinnula*, and about 2.1 to 2.5 times in *Rexea, Promethichthys, Mimasea* and *Gempylus*.

Abridging above, it is considered that in fusiform genera, e.g., *Lepidocybium* and *Ruvettus*, the cranium is rather deep and broad, whereas in elongate genera, e.g., *Mimasea* and *Gempylus*, the cranium is much slender in form. Namely, with elongation of the body form, there is a definite tendency towards increase in preorbital

length and decrease in both depth and width of the cranium.

Discussion: As has repeatedly been mentioned, the cranium of the gempylid fishes treated here shows gradation from the ordinary scombroid type to extremely slender one in correlation to the body form. Based upon the form of the cranium, the genera of the gempylids can be roughly divided into two major groups, *Lepidocybium*, *Ruvettus*, *Epinnula* and *Neoepinnula* on the one hand and *Rexea*, *Promethichthys*, *Mimasea* and *Gempylus* on the other. The cranium is much narrower and lower, and the preorbital region is considerably longer in fishes belonging to the second group than in those referred to the first one. Especially attention should be directed to the remarkably projected preorbital portion of the cranium in *Mimasea* and *Gempylus*. It is quite possible that the unusually elongated preorbital portion of the cranium has a large share in the formation of the acutely pointed conical snout in the fishes of these two genera.

Of particular interest among the gempylid craniums is the posterior extension of pterotic region in both *Mimasea* and *Gempylus*. A similar structure is shown by STARKS (1911) in *Lepidopus*, a member of the family Trichiuridae, in which the posterior edge of the pterotic is projecting backward and sharply pointed. This similarity as well as a close resemblance of general appearance of the cranium, may suggest that these genera, especially *Gempylus*, are closely related to the trichiurids.

STARKS (1911), working with *Promethichthys*, presented, as one of the osteological characteristics of the family Gempylidae, the fact that the opisthotic does not separate the exoccipital from the pterotic in view of the dorsal surface. But so far as our examinations go, this evidence is only found in *Rexea* and *Promethichthys*. Accordingly, it is unlikely to place the pterotic-exoccipital suture in the osteological feature of Gempylidae.

A certain peculiar feature is found in *Mimasea* cranium, where the parietal is not ridged but flattened, the opposite is the case with the bone which usually bears a longitudinal crest in all the other fishes belonging to the family Gempylidae. Furthermore, in *Mimasea*, the epiotic crest is rather low and showing different appearance from the bone of the other members.

5. Shoulder girdle

Description: The shoulder girdle is composed of seven well ossified skeletal elements such as posttemporal, supraclavicle, clavicle, hypercoracoid, hypocoracoid, actinost and postclavicle. There is no mesocoracoid. The general arrangement of these elements is virtually same in all species herein examined (Fig. 7).

The uppermost and key suspensory bone of the shoulder girdle is the posttemporal. It is widely forked anteriorly, the dorsal branch of which is much longer than the ventral one. The upper branch extends upward and forward and solidly fits the epiotic by a ligament. The lower one extends ventro-anteriorly and conjoins with the posterior edge of the opisthotic. The ventro-posterior portion of the bone overlies the upper edge of the supraclavicle. There exist no generic difference in the structure of

this bone, but in *Lepidocybium* the posttemporal is rather broadly expanded and is unlike to that of the other member in general appearance (Fig. 7A).

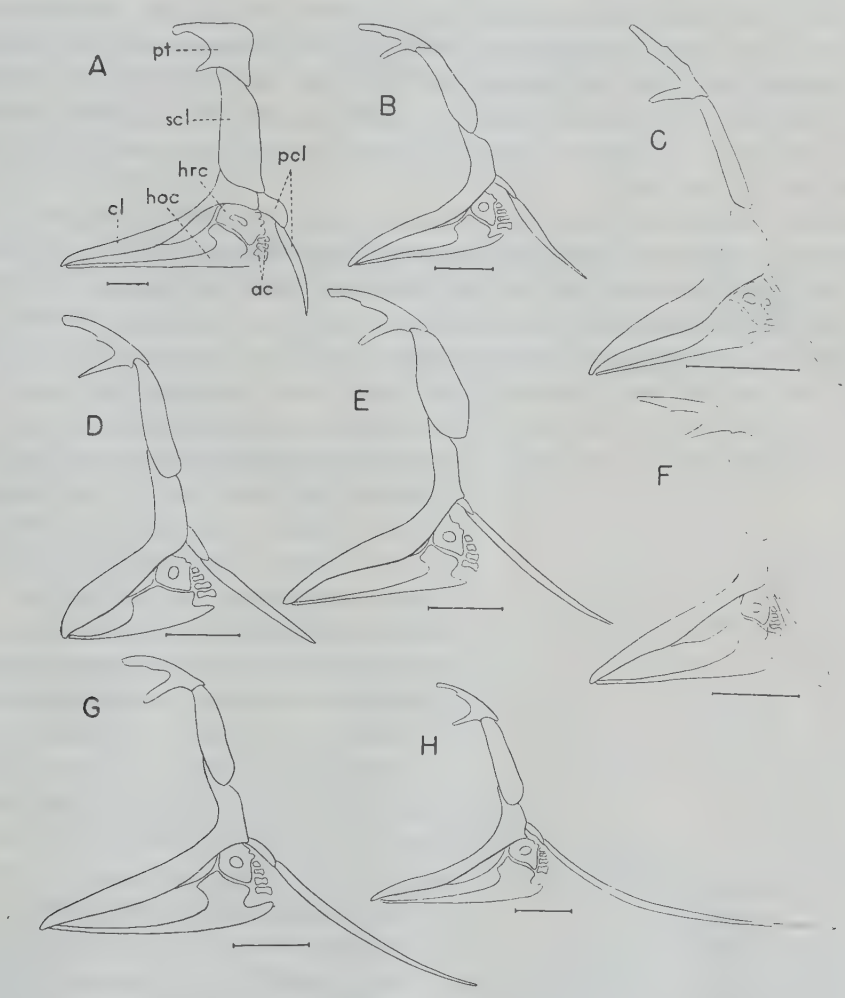


Fig 7. Lateral aspects of the shoulder girdle in 8 species of the Japanese gempylids. A, *Lepidocybium flavo-brunneum* ; B, *Ruvettus pretiosus* ; C, *Neoepinnula orientalis;* D, *Epinnula magistralis* ; E, *Promethichthys prometheus* ; F, *Rexea solandri* ; G, *Mimasea taeniosoma* ; H, *Gempylus serpens*. ac, actinost ; cl, clavicle ; hoc, hypocoracoid ; hrc, hypercoracoid ; pcl, postclavicle ; pt, posttemporal ; scl, supraclavicle. Each scale indicates 10 mm.

The supraclavicle is a simple, flat, slender bone extending from the ventro-posterior edge of the posttemporal to the upper portion of the clavicle. In *Lepidocybium*, *Promethichthys* and *Rexea*, the bone is rather broader than that of the other genera of this family (Fig. 7A, E. F). The upper edge of the bone underlies the posttemporal

and the lower portion largely overlies the anterior upper part of the clavicle.

The clavicle is a long bone of rather complex shape forming the strut of the lower portion of the shoulder girdle. Its upper end is attached to the inner surface of the supraclavicle. It runs downward and then extends ventro-anteriorly to the midline of the ventral side of the body, where it joins with the counterpart of the opposite side. In elongate forms such as *Mimasea* and *Gempylus*, the lower arm of the bone is greatly elongated and it is about quadruple as long as the upper arm (Fig. 7 G,H). Posteriorly the bone is attached to both the hypercoracoid and hypocoracoid near the posterior corner of the angle.

The hypercoracoid, in general, is briefly rhombic in shape lying below and behind the angle of the clavicle. It articulates with the hypocoracoid ventrally by an intervention of the cartilaginous band, and with the lower lamina of the clavicle anteriorly. The bone is pierced through by an ovoid foramen at the central part of the bone. In *Lepidocybium* this foramen is much elongated (Fig. 7 A). The upper half of the posterior border of the bone is thickened and bears two or three condyles resembling the actinost in general appearance.

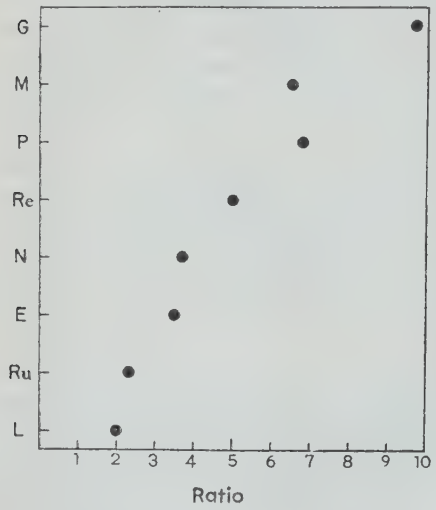


Fig. 8. Ratio of the posterior element of the postclavicle to the anterior element of the same in 8 species of the Japanese gempylids. The 8 species put on vertical axis are arranged merely in order with equidistance from thick form to elongated one. E, *Epinnula magistralis*; G, *Gempylus serpens*; L, *Lepidocybium flavobrunneum*; M, *Mimasea taeniosoma*; N, *Neoepinnula orientalis*; P, *Promethichthys prometheus*; *Re*, *Rexea solandri*; *Ru*, *Ruvettus pretiosus*.

The hypocoracoid is a thin falciform bone supporting the lower part of the shoulder girdle. The head of the bone is attached to the hypercoracoid dorsally and to the lower lamina of the clavicle anteriorly. The bone tapers ventro-anteriorly and reaches to the anterior extremity of the clavicle. The ventro-posterior corner of the bone is acutely projected posteriorly.

Usually there are four rod shape actinosts arranged in a single row behind the hypercoracoid. They are immovably attached to the posterior border of the hypercoracoid. In *Lepidocybium* the uppermost actinost is united by ankylosis with the hypercoracoid (Fig. 7A).

Two elements of the postclavicle extend in a continuously overlapped row from the posterior edge of the angle of the clavicle to behind the pectoral base. The anterior element is much smaller than the posterior one and attached to the clavicle anteriorly. It is invariably narrow except for *Lepidocybium*, in which the bone is rather broad and laminar in shape (Fig. 7 A). The posterior element, slender bayonet in shape, begins beneath the rear end of the anterior element and tapers ventro-posteriorly. There are some generic differences in the length of the posterior element of the postclavicle. In the elongate

species, for example, *Mimasea* and *Gempylus*, it is exceedingly elongated and about twice as long as the clavicle (Fig. 7 G, H).

Discussion : As will be recalled from the descriptions, the most striking feature of the shoulder girdle is the shape of the postclavicle. The comparative anatomy shows a good correlation between degree of elongation of the postclavicle and body form of the fish. The postclavicle is greatly elongated in elongate species, but not so elongated in fusiform species (Fig. 8). Of two elements of the bone, the elongation is occurred only in the posterior element. The anterior element is not so variable in relative length in different species.

TUCKER (1953) presented fine radiographs showing the skeletal elements of the pectoral region of 3 trichiurid fishes, *Lepidopus caudatus* (EUPHRASEN), *Benthodesmus simonyi* (STEINDACHNER) and *Aphanopus carbo* LOWE. Comparing these figures with those of the gempylids, one may be able to prove a similarity of the shape of postclavicle existing between these species and gempylid fishes, especially *Promethichthys*, *Mimasea* and *Gempylus*.

Lepidocybium is peculiar in providing the broad posttemporal and also broad anterior element of the postclavicle. Such modifications in the shoulder girdle are not recognizable in the other members of this family.

6. Pelvic girdle

The pelvic girdle consists of a pair of slender wedge-like bones and is merely embedded in the muscle of the ventral body wall. Each bone tapers anteriorly to terminate into a point. There is no generic difference essentially in the structure of the pelvic bone. But a reduction of skeletal element is met in some species having rudimentary pelvic fin. The following table gives a correlation of skeletal reduction of the pelvic girdle to the degree of development of the pelvic fin in some elongate

Table 2. Pelvic fin formula, degree of development and position of insertion of the pelvic fin in 8 species of the Gempylidae

Genera	Pelvic fin	Pelvic girdle	Insertion of pelvic fin
Lepidocybium	I, 5	Developed	Below the pectoral base
Ruvettus	I, 5	Developed	Below the pectoral base
Epinnula	I, 5	Developed	Below middle portion of the pectoral fin
Neoepinnula	I, 5	Developed	Below middle portion of the pectoral fin
Rexea	Single spine	Reduced	Below anterior end of the pectoral base
Promethichthys	Single spine	Slightly reduced	Below anterior end of the pectoral base
Mimasea	I, 5	Developed	Just behind perpendicular through posterior end of the pectoral base
Gempylus	I, 4 (Much reduced)	Reduced	Below posterior end of the pectoral base

species. This table also demonstrates the position of insertion of the fin. It is a matter worthy to be mentioned that in *Rexea*, *Promethichthys* and *Gempylus* the insertion

agrees with that of *Diplospinus* and *Aphanopus*, which are primitive members of Trichiuridae, ascertained by TUCKER (1956).

7. Caudal skeleton

Description : The forked caudal fin is supported by processes of the last three vertebrae. The last vertebra is composed of rather complex bony elements (Fig. 9).

The urostyle, usually a rod-shaped bone extending upward and backward, is indiscernible in gempylid fishes due to the complicated ankylosis of associated bony elements. There is a prominent lateral keel near the base of the urostyle.

The hypurals are also firmly joined each other and their boundaries are obscure. So they roughly form as a whole a fan-like bony plate in shape. The median portion of the posterior border of the hypural plate is strictly notched. Along the ventral margin of this notch the plate is divided into two parts, the upper and lower lobes, except for *Lepidocybium* in which the boundary is entirely absent (Fig. 9 A). Furthermore, the lower lobe is divided into three parts in *Epinnula*, *Neoepinnula* and *Promethichthys* (Fig. 9 C, D, E), and into two parts in *Ruvettus*, *Rexea*, *Mimasea* and *Gempylus* (Fig. 9 B, F, G, H). The upper lobe is further divided into two parts in *Ruvettus*, *Epinnula*, *Promethichthys* and *Mimasea* (Fig. 9 B, D, E, G).

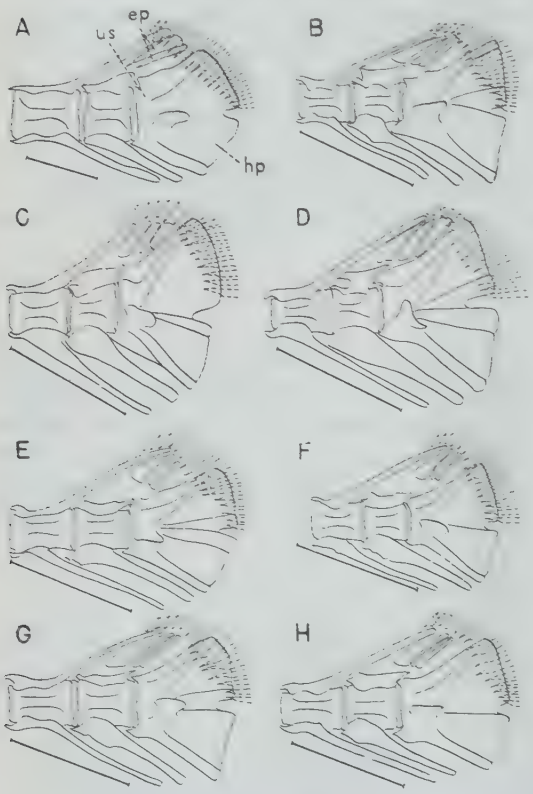


Fig. 9. Lateral aspects of caudal skeletons in 8 species of the Japanese gempylids. A, *Lepidocybium flavo-brunneum* ; B, *Ruvettus pretiosus* ; C, *Neoepinnula orientalis* ; D, *Epinnula magistralis* ; E, *Promethichthys prometheus* ; F, *Rexea solandri* ; G, *Mimasea taeniosoma* ; H, *Gempylus serpens*. ep, epural ; hp, hypural ; us, urostyle. Broken lines show insertion of upper half of caudal rays. Each scale indicates 10 mm.

Three slender rod-like epurals lie ranging above both posterior portion of penultimate vertebra and the dorso-anterior border of the urostyle. But *Lepidocybium* is provided with only two epurals (ep. in Fig. 9 A).

The last neural spine, attaching to penultimate vertebra, is greatly modified to lamina in shape. It overlies along the entire dorsal border of the vertebra. The last two or three vertebrae are not abruptly shortened as those of many other scombrids.

The bases of the caudal fin rays are not so deeply forked and sturdily attached to the distal portion of neural spines, epurals, hypurals and haemal spines. The hypurals are not so greatly overlaid by bases of caudal rays.

Discussion : According to REGAN (1909), MYERS (1932) and MUNRO (1949), a characteristic that is of greater utility in distinguishing the gempylid fishes is the depth of the basal divergence of caudal rays. MUNRO (1949), discussing in detail on this matter, said that the caudal rays are not so deeply forked at base in the gempylids, whereas the hypural bones are mostly hidden by the deeply forked bases of the caudal rays in other members of the Scombriforms. All species examined herein show undoubtedly gempylid type in this feature. No significant specific difference is observed in the basal divergence of caudal rays.

The posterior border of the hypural is deeply notched at median portion in all gempylids. Most of fishes belonging to the family Scombridae lack such a notch in hypural bone. It may be said therefore, that existence of such a notch will be of important feature together with the shallow basal divergence of caudal rays to distinguish the Gempylidae from the Scombridae.

In *Lepidocybium* the hypural bones are heavily ossified and strongly coalesced. This is quite a similar case with that of *Xiphias* described by GREGORY and CONRAD (1937).

Although STARKS (1911) said that *Promethichthys* lacks the lateral keel on the caudal skeleton, a well developed keel is always present at the basal part of the urostyle not only in *Promethichthys* but also in other members of gempylid fishes treated in this investigation (Fig. 9 A–H).

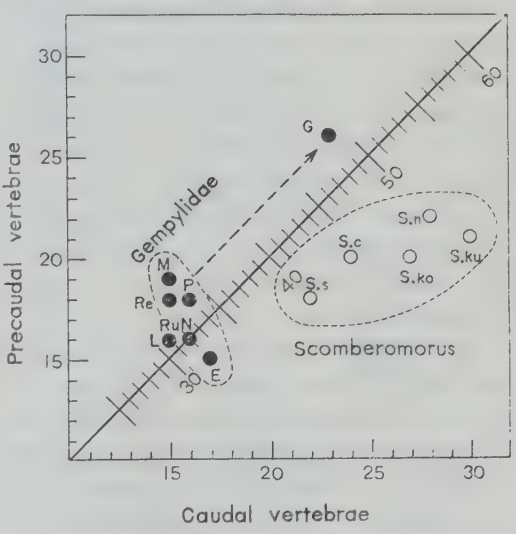


Fig. 10. Number of vertebrae in 8 species of the Japanese gempylids and five species of the genus *Scomberomorus*. E, *Epinnula magistralis*; G, *Gempylus serpens*; L, *Lepidocybium flavo-brunneum*; M, *Mimasea taeniosoma*; N, *Neoepinnula orientalis*; P, *Promethichthys prometheus*; Re, *Rexea solandri*; Ru, *Ruvettus pretiosus*; S.c, *Scomberomorus commerson*; S.ko, *S. koreanus*; S.ku, *S. kuhlii*; S.n, *S. niphonius*; S.s, *S. sinensis*. Graduation on a diagonal shows the total number of vertebrae in a respective species. Vertebral counts of fishes of the genus *Scomberomorus* are cited from data presented by KISHINOUYE (1923).

8. Number of vertebrae

Description: Although it is generally believed that the vertebral number of fish is influenced by the environmental factors such as water temperature and oxygen pressure during early developmental stages (TÁNING, 1946·1950), little intraspecific

variation is observed in such specialized teleostean fish as scombrids or serranids. Thus, it is sporadically accepted to make use of this feature for the separation of species. In order to make accurate interpretation, the counts of vertebrae are fully described below dividing into three items, total number, precaudal number and caudal number, of which latter two are put into a parenthesis. Each value of vertebral count extended herein includes the urostyle.

The vertebral counts of 8 species of the gempylids dissected in this study vary as a whole from 31 to 49. The counts of each of the Japanese gempylids are as follows: 31 (16+15) in *Lepidocybium*, 32 (15+17) in *Epinnula*, 32 (16+16) in *Ruvettus* and *Neoepinnula*, 33 (18+15) in *Rexea*, 34 (18+16) in *Promethichthys*, 34 (19+15) in *Mimasea*, and 49 (26+23) in *Gempylus*. Figure 10 gives a comparison of specific value of this characteristic between the family Gempylidae and the genus *Scomberomorus*. As is clearly shown in this figure, in the gempylid fishes, the number of precaudal vertebrae is somewhat greater than the number of caudal vertebrae with exception of some genera such as *Ruvettus*, *Epinnula* and *Neoepinnula*. Conversely, in all species of the Japanese *Scomberomorus*, the caudal vertebral counts always exceed the precaudal one. Attention may also be called to a curiously high value of the vertebral counts of *Gempylus* as compared with the other species of the family Gempylidae.

Discussion: Because of its variability, it will be wise to await further examinations on a sufficient number of specimens to establish the specific value of vertebral number. However, since the vertebral counts appear to be of significant characteristic in considering the relationship of scombrids, we would like to discuss this problem, using the data obtained in the present dissection.

Except for *Gempylus* the gempylid fishes range as a whole from 31 to 34 in vertebral counts. These values, especially those of relatively slender forms such as *Rexea*, *Promethichthys* and *Mimasea*, are unexpectedly low in comparison with the other group of Scombridae. According to FRASER-BRUNNER (1950), scombrids range as a whole from 38 to 64 in vertebral counts with a few exception of *Scomber* (31) or *Grammatorcynus* (31). In *Gempylus* the number of vertebrae is greatly increased (49). An extremely elongated body form of *Gempylus* may lead one to understand this remarkable result.

There is an urgent necessity for the consideration of the relationship between number of precaudal and caudal vertebrae. In gempylids the precaudal vertebrae counts slightly exceed, or are slightly less than the caudal vertebrae. This fact coincides well with the superficial body form,i.e., the trunk is apparently longer than the caudal region. In order to make comparison of this relationship with the other group of Scombrina, the vertebral counts of fishes of the genus *Scomberomorus*, which is considered to be one of the most closely related group among the family Scombridae to gempylids, are plotted in Figure 10. In *Scomberomorus* the counts of caudal vertebrae show higher value than that of the precaudal vertebrae. And these fishes exhibit relatively elongated caudal region in superficial aspect. It is of interest to note that although both *Gempylus serpens* and *Scomberomorus niphonius* bear

nearly same counts of total vertebrae, the number being 49 in the former and 50 in the latter, the precaudal-caudal relationship is virtually different between these two species, the vertebral formula being 26+23 in the former species and 22+28 in the latter. Figure 10 clearly suggests that an increase of vertebrae occurs in the precaudal region in gempylids whereas it occurs in the caudal region in scomberomorids.

One is tempted to regard the composition of the vertebral count in *Diplospinus multistriatus*, a stem-member of the family Trichiuridae. The description presented by TUCKER (1956) indicates that the vertebral count of this species is 58 (34+24). Namely, in this species the precaudal vertebrae also exceed the caudal vertebrae in number as is the case with the gempylids. This evidence may imply the close relationship of the gempylids with the trichiurids.

On the other hand it should be pointed out that most of the gempylids, especially *Lepidocybium*, bear similar vertebral counts to an archaic scomberomorid, *Cybium speciosum* (15+15=30) described by WOODWARD (1901), which is thought to be an ancestor of the scomberomorids.

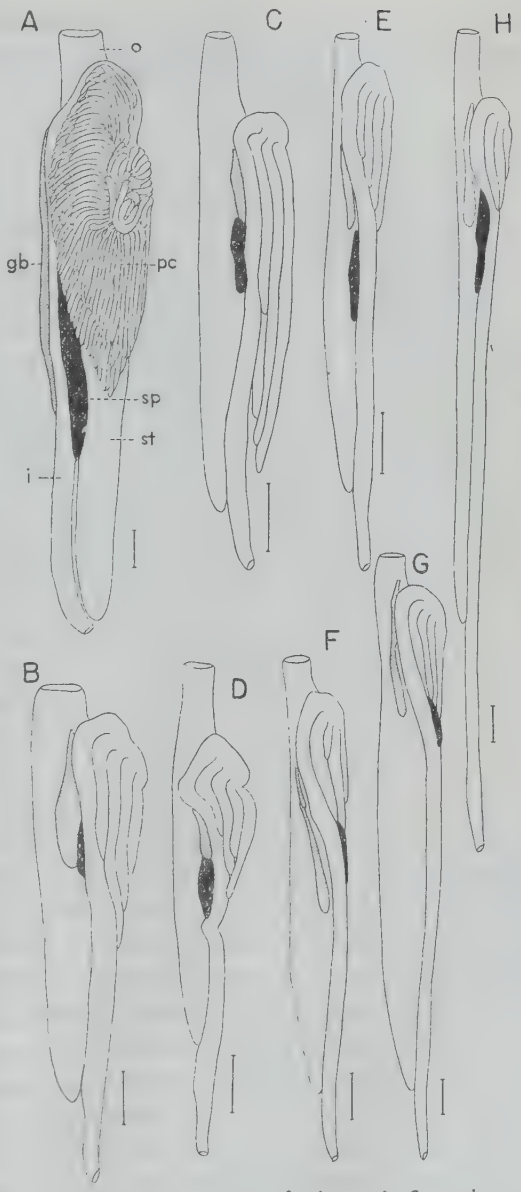


Fig. 11. Lateral aspects of viscera in 8 species of the Japanese gempylids. A, *Lepidocybium flavo-brunneum*; B, *Ruvettus pretiosus*; C, *Neoepinnula orientalis*; D, *Epinnula magistralis*; E, *Rexea solandri*; F, *Promethichthys prometheus*; G, *Mimasea taeniosoma*; H, *Gempylus serpens*. g.b, gall-bladder; i, intestine; o, oesophagus; p.c, pyloric caeca; sp, spleen; st, stomach. Each scale indicates 10 mm.

9. Alimentary canal and appendages

Description : The alimentary canal of the gempylid fishes is well differentiated as in many other teleostean fishes. The canal, as is usually

done, can be roughly divided in three regions, oesophagus, stomach and intestine. There is no generic difference in the fundamental structure of digestive system of the gempylids, although an elongation occurs in some organs of elongated species. The accessory organs of the digestive system are also well developed in all species examined.

The oesophagus, extending from the pharynx to the stomach, is a short muscular tube. Since it leads into the stomach without constriction, the demarcation between them is obscure. The stomach is a large blind sac of slender shape, and its posterior extremity reaches immediately before the anus. This extraordinarily developed stomach suggests that, like that of many other deep-sea fishes, it is capable of taking large or numerous prey. The intestine originates from the ventro-anterior surface of the stomach. It extends dorso-anteriorly to the anterior corner of coelomic cavity where it makes a turn posteriorly. Then it runs straight backward as far as the anus without convolution. The intestine is extending along ventral and right side of the stomach except for *Lepidocybium* in which it runs along the dorsal border of the stomach. The whole length of the intestine is nearly uniform in thickness.

The anterior part of the intestine emerging from the pylorus is surrounded by prominent pyloric caeca extending backward along the ventral and right side of the stomach. With exception of *Lepidocybium*, the caeca are considerably large in size. *Lepidocybium* bears fascicular mass of small pyloric caeca overlying the anterior regions of both the stomach and intestine on the right side and partially on the left side. Gempylids may be classified into three major groups by means of the number of caeca. The first type is represented by *Lepidocybium* bearing numerous caeca which could not be counted accurately (Fig. 11 A). *Ruvettus* is referred to the second type in having 15–16 caeca (Fig. 11 B). The third group contains all the other genera providing 7 to 11 caeca, the number of pyloric caeca being usually 8 in *Epinnula*, 9–11 in *Neoepinnula*, 7–9 in *Rexea*, 7–8 in *Promethichthys*, 8 in *Mimasea* and 9 in *Gempylus*. Above all, the pyloric caeca of *Neoepinnula* are of extremely large, being about three-quarters as long as the intestine (Fig. 11 C).

The gall-bladder is an elongated tubular organ, gradually tapering anteriorly. It rests on dorso-anterior surface of the intestine, and is closely associated with the right lobe of the liver. In *Lepidocybium* the organ is enlarged together with the spleen, being nearly half as long as the stomach (Fig. 11 A). In *Promethichthys* the organ is divided in two parts by a constriction (Fig. 11 F).

There is a large spleen near the dorsal surface of the pyloric caeca. It rests on the ventral border of the intestine in *Lepidocybium*, *Promethichthys* and *Mimasea* (Fig. 11 A, F, G), but on the dorsal surface of the intestine in other species.

Discussion: The fact that the gempylid fishes are provided with the deeply gaped mouth, stout canine-like teeth, reduced gill-rakers, large stomach extending as a slender blind sac and relatively short intestine may be enough to suggest that these fishes, as it is usual in most predatory deep-sea fishes, are a typical voracious feeder.

As is evident in Figure 11, *Lepidocybium* is distinguished from other genara of the Gempylidae by having numerous pyloric caeca, which are more than 200 in con-

trast fewer than 16 in other genera. *Lepidocybium* has, in addition to this distinctive feature, astonishingly elongated gall-bladder and spleen. In general appearance these organs resemble those of scomberomorid fishes illustrated by MUNRO (1943). Only a different point in digestive system of this fish from *Scomberomorus* is the intestine, which is simple straight canal instead of being considerable looped in the latter. These resemblances may suggest that *Lepidocybium* bears close relationship to the scomberomorids rather than the gempylids so far as the visceral characteristics are concerned.

According to the description presented by TUCKER (1956), the trichiurid fishes can be divided into three groups corresponding to subfamilies by the number of pyloric caeca. The number of pyloric caeca ranges from 6 to 9 in Aphanopodinae, reaches more than 20 in Lepidopodinae and shows more in Trichiurinae. An increment of caeca counts appears to serve as one of important characteristics in cosidering the intrafamily evolutionary trends of the trichiurids. Attention should be directed to the fact that, in either the number of pyloric caeca (7–11) or general physiognomy of the alimentary canal, most of the gempylids, with exception of *Lepidocybium* and *Ruvettus*, are similar to Aphanopodinae which are considered to be the primitive group of the family Trichiuridae. These similarities enable us to surmise a close relationship of the gempylids with the trichiurids. In this view a formidable number of pyloric caeca in *Lepidocybium* is too difficult to be recognized as an intrafamily variation.

IV. General discussion

1. Evaluation of the generic status of *Neoepinnula*

The genus *Neoepinnula* was established to accommodate *Epinnula orientalis* GILCHRIST and von BONDE in our previous paper (MATSUBARA and IWAI, 1952) based chiefly upon the external features. In spite of its distinguishable features which are of sufficient consistency to warrant the generic separation, GREY (1953) regarded this genus as synonymous with *Epinnula* and suppressed this generic name without giving any comment. In general it is believed that such problem can best be solved by an examination of the internal characteristics, particularly of skeletal system. Hence, in order to clarify the status of this genus, an exhaustive discussion is made below on various features of both *Neoepinnula* and *Epinnula*.

First we should regard the skeletal structure of posterior portion of the cranium and the position of the anterior interneural spines. A noteworthy difference in the posterior portion of cranium between these two genera is the fact that the posterior edge of supraoccipital crest is sharply elevated in *Neoepinnula*, while it is rather low in *Epinnula* (Fig. 6 A', B'). In both genera the interneural spines are slender triangular plates, but the anterior ones are inclined upward and backward in *Neoepinnula* the opposite being the case with *Epinnula*, in which they extend upward and forward. Furthermore, in *Neoepinnula* the first two interneural spines rests far above the space between the second and third neural spines, leaving a wide space between them and the cranium (Fig. 6 B'), but in *Epinnula* the first two interneural spines

are deeply rooted between bases of the first and second neural spines, the first interneural spine extends to the posterior edge of the supraoccipital crest, leaving no space between it and the cranium (Fig. 6 A'). It goes without saying that the position of the interneural spines is closely related to that of the dorsal fin. Thus, it may readily be apprehended that the dorsal fin originates behind the perpendicular through the upper angle of the gill-opening in *Neoepinnula*, whereas it begins above posterior margin of the preopercle in *Epinnula*.

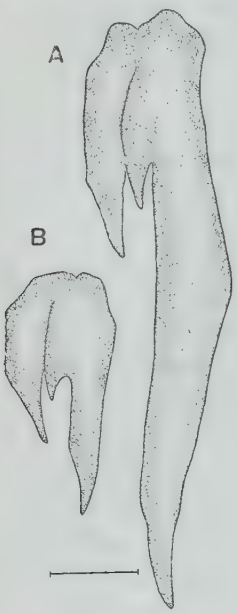


Fig. 12. Ventral aspect of liver in *Neoepinnula orientalis* (A) and *Epinnula magistralis* (B). Scale indicates 10 mm.

Further striking points of differences in the internal features of *Neoepinnula* from *Epinnula* are extraordinarily well developed liver and pyloric caeca. As illustrated in Fig. 11, the pyloric caeca are so long that their posterior ends reach shortly before the vent in *Neoepinnula*, while they are rather short and fail to reach midway to the vent in *Epinnula*. The liver of *Neoepinnula* is formidably developed and is more than three times as large as that of *Epinnula* (Fig. 12). Such hypertrophied liver as in *Neoepinnula* is characteristic of most of the deep-sea fishes. As well as this evidence the following distinctive features of *Neoepinnula* appear to be suffici-

Table 3. Comparison of certain characteristics in *Neoepinnula* and *Epinnula*.

Items	*Neoepinnula*	*Epinnula*
Lateral line	Originate simultaneously at upper angle of gill-opening	Bifurcating below membrane between fifth and sixth dorsal spines
Lateral line scales	Provided with 5 to 7 irregular hollows along both dorsal and ventral margins	Perforated by a small ovoid pore near center
1st dorsal spine	Flexible, and inserted behind upper end of gill-opening	Stout, and originated above posterior border of preopercular margin
Pelvic fin	Slightly reduced, 2.4 to 3.3 times in head length	Well developed, 1.3 times in head length
Gill-raker at angle	Armed with biserial minute spines on its inside	Not armed with spine
Posterior part of supraoccipital crest	Sharply elevated	Not so elevated
1st interneural spine	Inserted above 2nd neural spine, and separated far from the cranium	Inserted between 1st and 2nd neural spines, and attached to the cranium anteriorly
Vomer	Toothed	Edentulous
Pyloric caeca	9–11, greatly enlarged	8, moderate in length
Liver	Greatly enlarged	Moderate in length

ent diagnostic to justify recognition of this species as distinct deep-sea habitant : (1) Brittle skeletal elements, (2) Flexible dorsal spines, (3) Considerably reduced ventral fin, and (4) Bluish brown body coloration.

There are more fundamental differences between these two genera, inclusive of the external features as already treated by us in a previous report (MATSUBARA and IWAI, 1952). These salient points of differences are summarized in Table 3.

The generic concepts may vary in certain extent by each systematist. Accordingly one may admit these differences as specific criterion, rather than generic value. We however are of opinion that these differences are sufficient to isolate *Neoepinnula* as a monotypic genus rather than regard this genus as synonymous with *Epinnula*, because in such specialized teleost as Scombrina, few specific difference, as a rule, can be recognized in the internal features.

2. Some consideration on phyletic pedigree of the Gempylidae

Inspecting the available data obtained in the present investigation and our previous study (MATSUBARA and IWAI, 1952), we have drawn Figure 13, which shows the generic relationships within the family Gempylidae, although these genera by no means show the progressive modifications that they have undergone during evolution. As is indicated in this figure, the gempylids are divided into two major groups, Lepidocybiinae and Gempylinae.

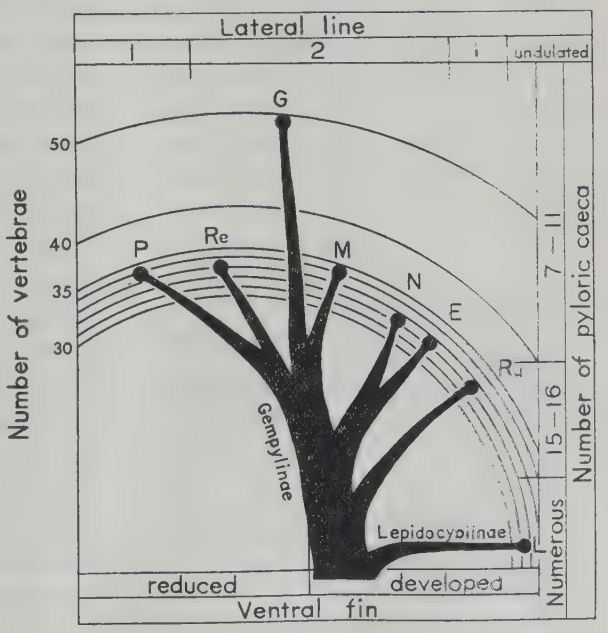


Fig. 13. Suggested phyletic pedigree of the Japanese gempylids inclusive of subfamilies and genera. Abbreviations are same as in Fig. 8.

The subfamily Lepidocybiinae is represented by a single genus *Lepidocybium*. *Lepidocybium*, which is placed at the base of the gempylid-stem, has been removed from the family Scombridae by MUNRO (1949) with following reasons : (1) In this genus the notching of the basal parts of caudal rays is very shallow as in members of Gempylidae, while it is usually deeply forked in those of Scombridae. (2) The dentition is canine-like. In addition to these two features, the fact that the posterior border of the hypural plate is deeply notched at the median portion, would strongly suggest that *Lepidocybium* does not belong to the family Scombridae. *Lepidocybium*, further, bears a few more points which resemble the gempylids. Although KISHINOUYE (1926) reported nothing

of gill-rakers in *Lepidosarda retigramma*, which is of a synonym of *L. flavo-brunneum*, gill-rakers are small and mostly hidden under the skin as in other gempylids. On the other hand, *Lepidocybium* shows disagreement with the other members of the family Gempylidae in providing the following features: (1) Numerous number of the pyloric caeca, (2) Unusually broad posttemporal, (3) Weakly developed fangs on the tip of upper jaw, (4) Well developed caudal peduncular keel, and (5) Undulated lateral line. Particularly in the characteristics of the structure of digestive system, position of lateral line and peduncular keel, this genus exhibits greater resemblance to the genus *Scomberomorus*. But its low vertebral count (16+15=31) is separated far from the range of that of *Scomberomorus* (40–51). In this feature, *Lepidocybium* resembles the *Grammatorcynus* which has 31 vertebrae. The possession of these peculiar features and certain other general resemblances of the skull, as well as the superficial body form, are evidence that *Lepidocybium* is closely related to *Scomberomorus* or *Grammatorcynus*.

Lepidocybium flavo-brunneum has been repeatedly plied between the family Scombridae and Gempylidae. This species was referred by SMITH (1849) to the genus *Cybium*, KISHINOUYE (1926) placed this species in the family Cybiidae under the name of *Lepidosarda retigramma*. WAITE (1904) and MYERS (1932) referred *Xenogramma carinatum*, which is a synonym of *L. flavo-brunneum*, to the family Gempylidae. MUNRO (1949) presented a decision to this problem that this species should be included in the family Gempylidae. Taking the feature of the peduncular keel into consideration, KAMOHARA (1938) separated this species from the other members of the gempylids.

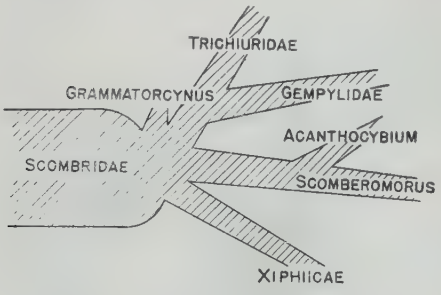


Fig. 14. Suggested phylogenetic relationship of the family Gempylidae and its closely related representatives.

As already stated above and as was pointed out by MUNRO (1949), it is highly probable that *Lepidocybium* is related to *Scomberomorus* and *Grammatorcynus* and through them to the scombrids. Accordingly, it is reasonable to assume that *Lepidocybium* represents an earliest offshoot that sprung off from the gempylid stock. These results are of sufficient magnitude to merit the retention of a separate subfamily Lepidocybiinae.

The subfamily Gempylinae, comprising majority of the gempylids, is a considerably complicated group. In general, among the members of the Gempylinae, it is possible to recognize some evolutionary trends in such features as presented in following paragraphs:

(1) Elongation of body form. (2) Projection of the snout. (3) Elongation of the cranium. (4) Increment of the preorbital space of the cranium. (5) Elongation of the posterior element of the postclavicle. (6) Multiplication of the vertebral counts. (7) Reduction of the pelvic fin and pelvic girdle. (8) Decrease in the number of pyloric caeca. On the basis of these important features and some other minor ones,

the intrasubfamily relationships would be discussed in detail.

Its general characteristics such as the fusiform body type, the single straight lateral line, the relatively large number of pyloric caeca (15–16), the low vertebral counts (32), the broad and deep cranium, the shortened preorbital space of the cranium and the short posterior element of the postclavicle, would lead one to suppose that *Ruvettus* is a very undifferentiated genus among the subfamily Gempylinae. It resembles more closely *Lepidocybium* in brief superficial form rather than the other members of the Gempylinae. That this fish is provided with well developed fins and is frequently captured by tuna long lines together with *Lepidocybium*, is suggesting that this fish is similar to *Lepidocybium* in habitation, as well, which perhaps is bathypelagic region. On the other hand *Ruvettus* is highly specialized in the condition of scalation. The skin of this fish is spiniferous caused by the tridentate scales covering the body surface.

The second divergence from the main branch leads us to two genera, *Epinnula* and *Neoepinnula*. They are characterized by having such features as the vertebral counts are 32 in average, and a tendency toward elongation has not yet occurred in body form, cranium, and other skeletal elements. These two genera, however, are considerably specialized toward unique direction, at least, in having the following characteristics: (1) The body is not elongated but is highly compressed laterally, (2) The lateral lines are two on each side, the lower branch extends along the ventral contour of the body, and (3) The pyloric caeca are reduced to 8 to 11 in number.

Since the genera, *Epinnula* and *Neoepinnula* are very closely related, it is difficult to trace the evolutionary trends between these two genera. As is generally believed, the most generalized group of Scombrina appear to be surface or midwater dweller. In general, the gempylids are phyletically considered as a group that migrated into deep water layer from the surface stock. It is evident that *Neoepinnula* inhabits deeper layer than *Epinnula* at least in providing the inadequate ossification, the relatively reduced pelvic fin, and extraordinarily well developed liver and pyloric caeca, all of which are of adaptative features to the deep-sea life. In this view, it may be able to say that *Neoepinnula* has been derived from an ancestor not very unlike *Epinnula*-like stock.

There are some similarities between *Epinnula* and *Grammatorcynus* in the position of lateral lines and vertebral counts. However, in view of the absence of data concerning the internal features of *Grammatorcynus*, it will be necessary to await further examination of the latter fish to discuss their relationship.

The branch leading to the genera *Rexea* and *Promethichthys* is peculiar in possessing so reduced pelvic fin which is represented by a single spine or entirely absent in adult. Of particular interest is the fact that in both genera, the pelvic spine becomes shorter with growth of the fish. It is already entirely absent in specimens larger than about 240 mm. in standard length in *Rexea*, but it is recognizable as a rudiment in specimens larger than about 500 mm. in *Promethichthys*. Furthermore, these genera show several other specialized features, i.e., the body is moderately elongated, the depth being contained less than 9 times in standard length,

the dorsal and anal fins are followed by 2 finlets posteriorly, the cranium is considerably elongated, especially in frontal and preorbital regions, the opisthotic does not separate the exoccipital from the pterotic in dorsal view, the posterior element of postclavicle is elongated, being 5 to 7 times as long as the anterior element, and the vertebral counts are slightly increased, being 33–34 as compared to 32 in average in *Epinnula* and *Neoepinnula*.

In many features these genera are closely related. The most discriminative characteristic between *Rexea* and *Promethichthys* is the lateral line. As already aforementioned, *Rexea* has two lateral lines, the lower branch of which runs along the midline of lateral side of the body, while *Promethichthys* has single one. On the basis of its position the lateral line of *Promethichthys* looks like the double lateral lines of *Rexea* that the upper branch disappeared out of our sight. Perhaps *Promethichthys* lateral line is secondarily reduced type from the *Rexea* one (Fig. 2).

The fact that *Mimasea* and *Gempylus* are characterized by having the extremely elongated body, being 10 to 12 times as long as deep, the sharply projected snout, the elongated cranium, being about 4 times as long as deep, the greatly elongated posterior element of the postclavicle, being about 6.5–9.5 times as long as the anterior element, and the curious pterotic projection posteriorly, would lead us to believe these genera to be the most specialized line of evolution in the Gempylinae-stem. It is generally believed that this group is the most closely related gempylid to the trichiurids. Despite of its specialized characteristics, *Mimasea* retains yet relatively generalized features such as well differentiated pelvic fin with 1 spine and 5 soft rays, and low counts of both median fin-rays and vertebrae. These, as well as the flattened parietal, may suggest that *Mimasea* has, contrary to expection, no especially close relationship to the trichiurid fishes.

The genus *Gempylus* seems to be a highly specialized offshoot among the members of the Gempylinae-stem, and stand very close to the primitive trichiurids. In this genus, the vertebral counts are greatly increased up to 49 (26+23) or more, 53 (28+25) (REGAN, 1909), the posterior edge of the pterotic is projected as in *Lepidopus* described by STARKS (1911), and pelvic fin is so reduced to vestigial one spine and 4 soft rays. In vertebral feature it especially approaches, not only in total number but also in precaudal-caudal relationship, to that of a primitive trichiurid, *Diplospinus* in which the formula of vertebral counts is 34+24=58. *Gempylus*, however, has certain contradictory characteristics to this line such as double lateral lines and well differentiated detached finlets. In spite of these rather peculiar features, the maintenance of advantageous internal features may probably indicate that *Gempylus* rests near the evolutionary line between Gempylidae and Trichiuridae, though TUCKER (1956) advocated that *Nesiarchus* is more close gempylid to the trichiurids than *Gempylus*.

V. Conclusion

From the description presented above, it is obvious that the gempylids are composed of quite a small number of isolated forms. Indeed, as they vary rather ex-

tensively both externally and internally within a few species, it is, of course, arbitrary to try to trace a continuous track that they have undergone during evolution. Nevertheless, however, purely hypothetical it may be, we provisionally followed up certain evolutionary trends in some features found in this group. As is repeatedly regarded above, it is apparent that this family is closely related to certain groups of the family Scombridae such as *Scomberomorus* or *Grammatorcynus*. There is similar evident affinity between the Gempylidae and Trichiuridae. Accordingly it is reasonable to assume that the Gempylidae forms a well marked evolutionary line within the complex of the scombrid-trichiurid lineage (Fig. 14).

The results of the present comparative study of the Japanese gempylid fishes lead us to the conclusion that it is proper to recognize 9 genera and 9 species assigned into 2 subfamilies. A list of the systematic categories thus is arranged in the following paragraphs:

Subfamily Lepidocybiinae

Lepidocybium flavo-brunneum (SMITH)

Subfamily Gempylinae

Ruvettus pretiosus COCCO

Epinnula magistralis POEY

Neoepinnula orientalis (GILCHRIST and von BONDE)

Rexea solandri (CUVIER)

Promethichthys prometheus (CUVIER)

(*Nealotus tripes* JOHNSON)

Mimasea taeniosoma KAMOHARA

Gempylus serpens CUVIER

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Revision of *Brachytrichia* ZANARD. and *Kyrtuthrix* ERCEG.

By

Isamu UMEZAKI*

I. Introduction

A treatment of the genus *Brachytrichia* ZANARD., which was described on the basis of *B. rivularioides* by ZANARDINI in 1872, was included by BORNET & FLATAULT (1886) in their "Revision des Nostocacées hétérocystées"; two species, *B. Balanii* BORN. & FLAH. and *B. Quoyi* BORN. & FLAH., were placed in the genus. Since then the two species have been recognized by the majority of phycologists. On the basis of specimens by J. SCHMIDT, who collected at Koh Chang, gulf of Siam, GOMONT (1901) described as new *B. maculans*, which resembles illustrations of the young stages of *Hormactis Balanii* by BORNET & THURET (Notes Algologiques, 1880, Fasc. II, p. 172, pl. XLIII, f. 3, 4 & pl. XLIV, f. 1–3). *Brachytrichia affinis* SETCH. & GARDN., which resembles *B. Quoyi*, was added to the genus by GARDNER (1918). SETCHELL (1926) published a species endophytic in *Codium* plants, *B. Codii* SETCH. FRÉMY (1932) described *B. Balanii* f. *purpurea* FRÉMY, which grew in *Codium* plants. Thus, during half a century between 1886 and 1932 the genus *Brachytrichia* accumulated three species and a form in addition to *B. Balanii* and *B. Quoyi*.

The genus *Kyrtuthrix* ERCEG. was decribed by ERCEGOVIĆ (1929 a) on the basis of *K. dalmatica* ERCEG., a name which was transferred to *Brachytrichia* by FRÉMY (1934) as *B. dalmatica* (ERCEG.) FRÉMY. In the present work *B. maculans* GOM. is shown to be an earlier name for the species described by ERCEGOVIĆ.

GEITLER (1932) revised the genus *Brachytrichia* without reference to the type specimens, although WITTROCK & NORDSTEDT's Alg. exsicc. nos. 865 and 1197, HAUCK & RICHTER's Phyk. univ. no. 681, and COLLINS, HOLDEN & SETCHELL's Phyc. Bor.-Amer. no. 8 were examined; he placed all the species of *Brachytrichia*, except FRÉMY's *B. Balanii* f. *purpurea*, under the one species *B. Balanii* (LLOYD) BORN. & FLAH. At the same time, he classified *Brachytrichia* and *Kyrtuthrix* as separate genera respectively in the Mastigocladaceae. FRITSCH (1945) stated that it is not certain that BORNET & THURET dealt with only a single species, and he treated *Brachytrichia* and *Kyrtuthrix* as separate genera in the Scytonemataceae, subfamily Brachytrichieae.

I also had a doubt as to whether BORNET & THURET dealt with only a single species, as pointed out by FRITSCH, because certain plants reproduced in BORNET & THURET's illustrations closely resemble those of *Brachytrichia maculans* and *Kyrtuthrix dalmatica*. In solving the question I (1955) made a developmental study of the frond of *Brachytrichia Quoyi* and reached a conclusion that the young form of *B.*

* Botanical Laboratory

Quoyi differs from those of BORNET & THURET's illustrations and from those of *B. maculans* and *K. dalmatica* and that BORNET & THURET's materials must have been of another taxon as hinted by FRITSCH.

The present paper deals with a revision of *Brachytrichia* and *Kyrtuthrix*, on the basis of the original and other specimens assembled from all over the world.

II. The method of branching

BORNET & THURET (1880) described the mode of branching in *Hormactis Balanii* (LLOYD) THUR. and figured the developmental stages, mainly on the basis of material found in *Nemalion* (see Notes Algologiques, Fasc. II, p. 172, pl. XLIV, f. 3–8), and pointed out that the branching differs from that of either *Scytonema* or *Mastigocoleus*. BORNET & FLAHAULT (1886) described also the method of branching in *Brachytrichia* as sometimes *Scytonema*-like and sometimes composed of a single apical and two divergent basal portions. SETCHELL & GARDNER (1919) stated that the branching of the trichome is similar to that of *Mastigocoleus*, *Nostocopsis*, and *Herpyzonema*, which are true branching, and they observed that in *Brachytrichia* the branching is formed after the loop-formation or without the formation of loops which is realily true branching. Their plate 7, figure 28 shows no doubt true branching. Then they removed from the Rivulariaceae the genus *Brachytrichia* and placed it in the Stigonemataceae. ERCEGOVIĆ (1929 a) pointed out that the branching in *Kyrtuthrix* is similar to that of *Scytonema* in regard to loop-formation, but in the former the loop never breaks into two filaments. Moreover, he (1929 b) explained precisely the differences in branching of *Brachytrichia* and *Kyrtuthrix*. GEITLER (1925, 1932) named the method of branching in the Mastigocladaceae (including *Brachytrichia*, *Kyrtuthrix*, *Mastigocoleus*, and *Herpyzonema*) "V-Verzweigung." FRITSCH (1945) described in his book, "The Structure and Reproduction of the Algae," vol. II, p. 846, that the development of geminate branches by loop-formation as in *Scytonema* is met with in the Brachytrichieae and explained the method shown in ERCEGOVIĆ's figures: in *Kyrtuthrix* the two limbs of the loop often remain closely approximated, while in *Brachytrichia* (and *Herpyzonema*) one of the two limbs continues to segment so that a single branch surmounts the basal V-shaped portion.

Thus, plants of both *Brachytrichia* and *Kyrtuthrix* form loops (GEITLER's "Schlingenbildung") initially: in *Kyrtuthrix* the branching consists only of loop-formation, while in *Brachytrichia* a branch issues at the apex of the loop or the loop divides into two filaments at the apex as in *Scytonema*.

IYENGAR & DESIKACHARY (1953) reported branches with three-pored heterocysts in Indian material of *Brachytrichia Balanii*, and they (1954) issued their opinion on the development of branching in *Brachytrichia*: that a normal branch is formed first and then a V-shaped notch is developed below it, and that the mode of branching is in no way the same as the false branching in *Scytonema* or that of ERCEGOVIĆ's observation in 1929.

On the developmental process of branching in *Brachytrichia* there are two dif-

ferent theories, EECEOVIĆ's theory (1929 a, b) and IYENGAR & DESIKACHARY's (1954). But in the present study I have known that the mode of the branching in *Brachytrichia* is not confined to either of the two; sometimes the loop is formed first and then a branch issues at the apex, and sometimes the branch issues first and then the loop is formed, or as observed by SETCHELL & GARDNER branches develop without loop-formation, especially in the erect filaments, or sometimes geminate branches as in *Scytonema* are formed in the erect filaments.

I reported a new fact in my previous paper (1955) that in *Brachytrichia Quoyi* the trichomes branch in the normal Y-shaped form during the young stage of the frond; when the frond is matured the erect filaments branch in an inverse Y-shape; and in that paper I introduced a new term "Y-form" instead of "V-form", which has long been used to describe the branching in *Brachytrichia* and *Kyrtuthrix*, because when the branch is completed, the shape of the branch is that of normal or inverse "Y".

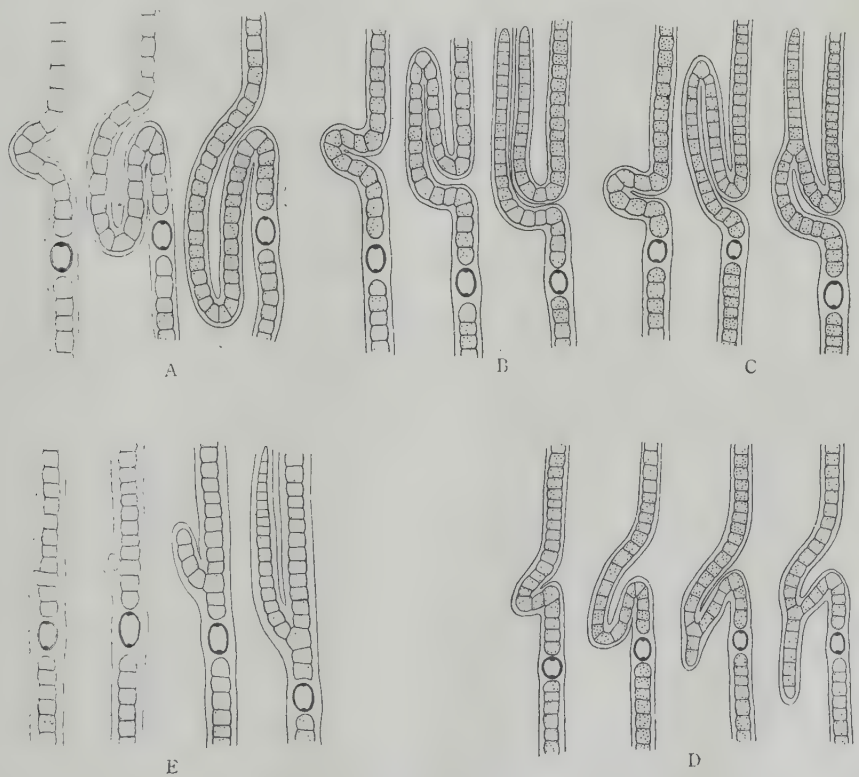

Fig. 1. Showing various forms of branching.
A, *Kyrtuthrix*: A, V-shaped branching. B–E, *Brachytrichia*: B, scytonematoid branching; C–D, Y-shaped branching, C, inverse Y-form; D, normal Y-form; E, branching without loop-formation.

In the present paper I have found that in *Brachytrichia* the formation of branching is various as mentioned above: without the formation of loops branches are laterally formed (true branching); after the formation of loops branches are formed,

or normal branches are formed first and then loops are developed below them (both: Y-shaped branching); and as in *Scytonema* after loop-formation the loop-shaped apex ruptures and geminate branches issue (scytonematoid branching). In *Kyrtuthrix* only loop-formation occurs throughout the life of the plant (V-shaped branching). (Figs. 1–2).

I observed many branches with three-pored heterocysts in the material of *Brachytrichia Quoyi* as described by IYENGAR & DESIKACHARY (1953).

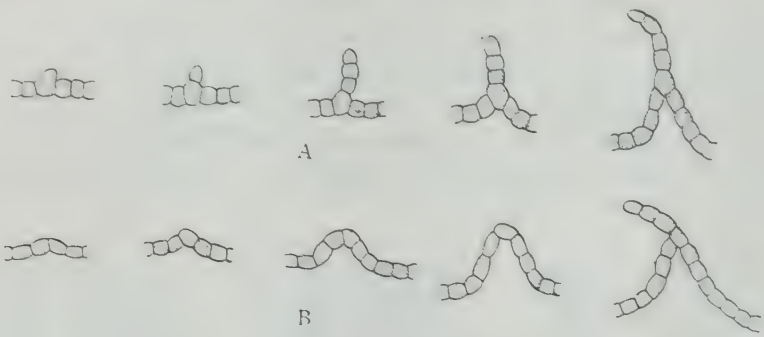

Fig. 2. Showing the development of Y-shaped branching.
A, a branch is formed first and then a loop is developed below it. B, after loop-formation a branch is formed.

III. Ecological notes

Habitat: – *Brachytrichia Quoyi* has been found mostly on rocks and barnacles in the littoral belt, sometimes on large algae (i. e. on *Fucus* in Woods Hole and Falmouth, Massachusetts), rarely in the fronds of *Codium* (BORNET & THURET 1880; SETCHELL 1926; FRÉMY 1932; FELDMANN 1937). The types of *Nostoc Quoyi* AG. (collected on the Mariana Islands), *Brachytrichia rivularioides* ZANARD. (collected on Borneo), and *B. affinis* SETCH. & GARDN. (collected on the California coast), were found on rocks; and the type of *Rivularia Balanii* LLOYD (collected on Belle Ile, France) was found on barnacles. On the Japanese coast the species has been found growing on rocks in the littoral belt or near high tide level. From an ecological point of view, I do not think that there is a fundamental difference between rocks and barnacle shells (or other mollusc shells). Perennial and hard plants like *Fucus* appear to be natural host for the growth of *B. Quoyi*. Endophytic plants in *Codium* surely originate from plants growing on rocks or barnacles; the *Codium* plant is very suitable for the growth and germination of the reproductive organs (hormogones and spores) of the blue-green algae as well as of other kinds of the marine algae.

Kyrtuthrix maculans has been found growing mostly on rocks in the littoral belt, rarely perforating calcium carbonate (ERCEGOVIĆ 1929 a), or rarely on mangroves (COLLINS & HERVEY 1917). On the Japanese coast the species has been found only on rocks in the littoral belt or in the supralittoral belt, but the perforating form has never been found. Compared with that of *Brachytrichia Quoyi* the character of the

habitat of *Kyrtuthrix maculans* is not so variable.

Size of frond : - The frond of *Brachytrichia Quoyi* is very variable in size, ranging from microscopical to macroscopical (up to 5 cm. or 7 cm. in diameter). SETCHELL & GARDNER (1919) reported that Woods Hole specimens were sometimes 7 cm. in diameter. Among the specimens of WEBER-VAN BOSSE's Siboga-Expedition the largest is 5.5 cm. broad. OKAMURA (1915, 1936) and FAN (1953) reported that the specimens in Formosa are 5 cm. or more in size. However, the type specimens are rather small. They are as follows: *Nostoc Quoyi* AG., 7 mm. in diameter; *Brachytrichia rivularioides* ZANARD., 7–12 mm. in diameter; *Brachytrichia affinis* SETCH. & GARDN., 3–3.5 mm. in diameter; and *Rivularia Balanii* LLOYD, 3–4 mm. in diameter. In Europe, especially on the Atlantic coast, this species is common, but the size of the frond is small, being up to 6 mm. in diameter. This species from the European coast has long been known under the name of *Brachytrichia Balanii* BORN. & FLAH. Considering from the standpoint of geographical distribution, the species is of subtropical or tropical rather than of temperate or subtemperate origin. As mentioned above, in subtropical or tropical regions it is very widely distributed and the frond is larger. This alga also grows commonly on the Japanese coasts, from the Amami Islands (Amami-oshima island) to Hokkaido (Muroran) -from ca. lat. 28° N. to ca. lat. 42° N. Geographically in the warmer regions such as the Amami Islands, Kyushu, and Shikoku it is abundant and luxuriant, while in the colder, northern districts such as Hokkaido and Matsushima Bay it is rare and the size of the frond is small, up to 1 cm. broad. The fronds collected from Amami-oshima island are mostly 1–2.5 cm. broad. In my collections the fronds from the warmer regions are generally larger than those from the colder, northern regions.

The fronds of *Kyrtuthrix maculans* is usually plane and sometimes widely expanded, up to 10 cm. or more broad, mostly on rocks or stones. The height of the frond is sometimes variable, 100–400μ, rarely 500μ. But, geographically there is no difference on the height.

Shape of frond : - The shape of the frond of *Brachytrichia Quoyi* is variable, cushion-shaped to spherical or bulbose, deeply or slightly plicated, or smooth on the surface.

In 1952 I made an ecological study* on *Brachytrichia Quoyi* at the Shirahama coast of Kii Peninsula, in which I observed that fronds with well plicated surface and fronds with smooth surface grew during the same season and in the same habitat. The smooth plants grew generally on rocks whose surfaces were exposed to direct sunlight during ebb tide, while the well plicated plants grew generally on rocks whose surfaces were immersed in tide pools or in rock depressions even during ebb tide. According to UTINOMI'S (1950) classification of marine biotic communities the former plant is a xerophilic epibiont and the latter a hydrophilic epibiont.

The frond of *Kyrtuthrix maculans* is always plane as that of *Isactis plana*, very

* This study was carried out with Dr. H. Utinomi's help.

firmly adhering to rocks, sometimes widely expanded, or rarely perforating calcareous rocks even when well matured.

Colour of frond : – Hitherto it has been the general opinion that *Brachytrichia Balanii* has brownish green fronds and that the fronds of *Brachytrichia Quoyi* are of a deep blue-green colour. It it well known that the colour of the blue-green algae shows a great variation. LINDSTEDT (1943) studied the colour of various species in culture and reported it is very changing with changes in the enviroment. I also noted on the Shirahama coast in 1952 that most of the xerophilic epibiont forms of *Brachytrichia Quoyi* were deep blue-green in colour, while the almost hydrophilic epibiont forms were brownish in colour.

The colour of *Brachytrichia Quoyi* in *Codium* is variable also. The plants in WITTR. & NORDST., Alg. exsicc. no. 865 b(L) are blue-green ; and according to FREMY's diagnosis those of *Brachytrichia Balanii* f. *purpurea* are purple. Some of my own collections are blue-green and some purple. The genus *Codium* contains many species. According to species and even in the same species their vertical distribution in the sea is very variable. LINDSTEDT (1943) and many other investigators reported that species inhabiting deep water or ones cultured under weak light possessed red or violet coloration. Thus it is natural to assume that the colour of *Brachytrichia Quoyi* endophytic in *Codium* is variable.

The frond of *Kyrtuthrix maculans* is deep- or blackish-blue-green, but it changes to nearly black when dried. The colour of the frond is not as variable as that of *Brachytrichia Quoyi*, because the habitat type is that of the xerophilic epibiont.

IV. Reproductive organs

In the genus *Brachytrichia* the hormogones are usually formed in the apical portions of the filaments, in the same way as that in many Rivulariaceae, and they are not uncommonly produced when the plant is matured. No other reproductive organ has been found in plants of this genus.

In the diagnosis of *Brachytrichia maculans*, GOMONT (1901) described the hormogones as up to 70μ long. On the other hand, according to ERCEGOVIĆ (1929 a), they are 25–40μ long in *Kyrtuthrix dalmatica*. The hormogones are not figured in GOMONT's illustration (pl. 5, fig. 6). ERCEGOVIĆ in his text-figure (fig. 3 b) shows a filament with a hormogone which is just passing out of the sheath. In the original specimen of *Brachytrichia maculans* GOM. I observed hormogones which are set free from the extremities of the filaments, as in *Brachytrichia Quoyi* and many Rivulariaceae, though they were not abundant. WEBER-VAN BOSSE said in her Siboga-Expedition report (1913) that, in spite of abundant material, hormogones were not found on the filaments ; and she added that conidial cells, which were divided vertically, were abundantly found. I also did not observe the hormogones in WEBER-VAN BOSSE's material. I think that WEBER-VAN BOSSE's conidial cells were probably of a coccoid Cyanophycean, *Entophysalis deusta* (MENEGH.) DR. & DAILY. In the Japanese specimens, I have sometimes found spore-like cells : the apical cells of the

filaments are successively transformed into spore-like rounded cells with a diameter of 1.5-3μ, not dividing vertically as in WEBER-VAN BOSSE's description. That the reproduction of this species is effected by such a method in addition to hormogone-formation is possible to imagine, for the formation of a hormogone is quite rare. In the majority of the Japanese specimens there is no hormogone-formation. But in the specimens from only two localities, Tsuruga Bay, Fukui Prefecture (Herb. I. Umezaki no. 222) and Oshoro of Hokkaido (Herb. I. Umezaki no. 330), I observed numerous hormogones formed in the apical portions of the filaments, as in the case of *Brachytrichia Quoyi*.

V. Acknowledgments

The type and other specimens in the following herbaria and institutes, each of which is indicated by means of the following abbreviations, are studied: PC, Laboratoire de Cryptogamie, Museum d'Histoire Naturelle, Paris (Curator of algae, Dr. P. BOURRELLEY); L, Rijksherbarium, Leiden (Headcurator, Dr. J. TH. KOSTOR); UC, Herbarium, University of California, Berkeley (Curator of algae, Dr. G. F. PAPENFUSS); FC, Cryptogamic Herbarium, Chicago Natural History Museum, Chicago (Curator, Dr. F. DROUET); TSA, Herbarium, Florida State University, Tallahassee (Curator, Dr. C. S. NIELSEN); D, Herbarium of Francis Drouet, Chicago; SAP, Herbarium, Botanical Institute, Faculty of Science, Hokkaido University, Sapporo (Curator, Dr. Y. YAMADA); TFRI, Taiwan Fisheries Research Institute, Keelung (Mr. K. C. FAN); and IU, Herbarium of Isamu Umezaki, Maizuru.

Acknowledgments are made to the above-mentioned herbaria and their staffs for the loan of the types and other algal speimens. I also wishes to express his sincere thanks to Dr. ERCEGOVIĆ who sent me a specimen of *Kyrtuthrix dalmatica*. Thanks are also due to Dr. F. DROUET who gave me valuable advice and read this manuscript.

VI. Classification

1. Fronds cavernous, cartilagineous, or microscopic; branches scytonematoid, Y-shaped, or without loop-formation formed; sheaths mostly diffluent. ..... *Brachytrichia*
1. Fronds flat, firm; branches V-shaped; sheaths firm. ..... *Kyrtuthrix*

Brachytrichia

ZANARDINI, Mem. R. Istit. Veneto 17 : 152, 1872.-Type species : *B. rivularioides* ZANARD.

Hormactis THURET, Ann. Sc. Nat. VI. Bot. 1 : 382, 1875.-Type species : *H. Balanii* THUR.

Fronds minuta aut globoso-cava, filis in gelatino nidulantibus farctis, flexuoso-curvatis, ad superficiem parallelis, sursum attenuatis, ramosissimis; ramis scytonemaceis, Y-formantibus et sine laqueis ramosis; vaginis junioribuss distinctis, tubulosis, demum

confluentibus obsoletis ; heterocystis intercalaribus sine ullo ordine positis ; multiplicatis hormogoniis.

One species :

Brachytrichia Quoyi (AGARDH) BORNET & FLAHAULT, Ann. Sc. Nat. VII. Bot. 4 : 373, 1886. *Nostoc Quoyi* AGARDH, Syst. Algar., p. 22, 1824. *Hormactis Quoyi* BORNET in FARLOW, Rep. U. S. Fish. Comm., p. 39, 1881. *Hormactis Farlowii* BORNET pro synon. in FARLOW, Rep. U. S. Fish. Comm., p. 40, 1881. –Type from the Mariana Islands, Pacific Ocean (PC).

Physactis Lloydii CROUAN, Florule du Finistere, p. 117, 1867. *Rivularia Balanii* LLOYD in CROUAN pro synon., loc. cit., 1867. *Hormactis Balanii* THURET, Ann. Sc. Nat. VI. Bot. 1 : 382, 1875. *Brachytrichia Balanii* BORNET & FLAHAULT, Ann. Sc. Nat. VII. Bot. 4 : 372, 1886.–Type from Belle Ile, France (PC).

Brachytrichia rivularioides ZANARDINI, Mem. R. Istit. Veneto 17 : 153, 1872.–Type from Sarawac, Borneo (L).

Brachytrichia affinis SETCHELL & GARDNER in GARDNER, Univ. Calif. Publ. Bot. 6 : 475, 1918.–Type from Laguna Beach, Orange County, California (UC).

Brachytrichia Codii SETCHELL, Univ. Calif. Publ. Bot. 12: 66, 1926.–Type from near Punaruu Pass, Tahiti, Society Islands (UC).

The type of the following has not been available for study ; its original description is here designated as the type until authentic material can be found :

Brachytrichia Balanii BORN. & FLAH. f. *purpurea* FRÉMY, C. R. A. S. 195 : 1413, 1932.

Fronds plicata vel plicato-expansa, minuta aut magna, ad 5(–7) centimetra crassa, atro-fuscescens vel atro-viridis, trichomatibus superioibus 5 6μ crassis, inferne laxius complicatis, flexuoso-curvatis, ad apicem in pilo longo attenuatis, ad genicula constrictis ; articulis valde irregularibus, superne sphaericis, disciformibus vel diametro tri-plo brevioribus, inferne oblongis vel cylindricis ; heterocystis quam cellulis caeteris majoribus, sphaericis vel parum depressis ; hormogoniis intra vaginam singulis vel duobus, 20–25μ longis.

Habitat : growing on stones, rocks, barnacle shells, and algae in the littoral belt, or in *Codium* plants in tropical and temperate marine waters.

Specimens examined :

France : in the fronds of *Codium*, Banyuls, Roussilon, CH. FLAHAULT, 12 Oct. 1887 (as *Brachytrichia Balanii* in WITTR. & NORDST., Alg. exsicc. no. 865 b, L) ; on barnacles, Belle Ile, J. LLOYD, Sept. 1854 (Type of *Physactis Lloydii* CROUAN, as *Rivularia Balanii* LLOYD, PC, L) ; on barnacles, Cherbourg, A. LE JOLIS (as *Hormactis Balanii* in LE JOLIS, Alg. mar. de Cherbourg no. 272, L) ; on barnacles, Le Croisic, E. BORNET & C. FLAHAULT, 19 Sept. 1887 (as *Brachytrichia Balanii* in WITTR. & NORDST., Alg. exsicc. no. 865 a, L, D) ; on barnacles, Cherbourg, A. LE JOLIS, 20 Jul. 1875 (as *Hormactis Balanii* in Herb. A. LE JOLIS no. 2305, L) ; on rocks, Antibes, E. BORNET, May 1872 (as *Hormactis Balanii*, L, D).

Pacific Ocean : among the utricles of *Codium*, near Punaruu Pass, Tahiti, Society Islands, SETCHELL & PARKS, July 1922 (Type of *Brachytrichia Codii* SETCH., UC) ; on littoral rocks, Mariana Islands, GAUDICHAUD, 1823 (Type of *Nostoc Quoyi* AG., PC); on littoral rocks, Hawaii, Hawaiian Islands, W. A. SETCHELL 10128, 1 Jul. 1924 (L, D, FC) ; on littoral rocks, Honduras Bay, Puerto Galera, Mindro, Philippines, G. T. VELASQUEZ 1089, 27 May 1946(D) ;

on littoral rocks, Catadman Sound, Cuyo, Palawan, Philippines, G. T. VELASQUEZ 3020, 6 Jun. 1951 (D) ; on littoral rocks, Keelung, Formosa, K. C. FAN, 24 Apr. 1951 (TFRI, IU) ; on littoral rocks, Hoko island, Formosa, YAMADA (SAP).

Japan : **Amami-oshima island.** Gusuku, on littoral rocks, I. UMEZAKI 1724, 7 Apr. 1955 (IU) ; Koniya, on littoral rocks, I. UMEZAKI 1768, 1804, 9–11 Apr. 1955 (IU) ; Naze, on littoral rocks, I. UMEZAKI 1852, 12 Apr. 1955 (IU); Akakina, on littoral rocks, I. UMEZAKI 1871, 13 Apr. 1955 (IU). **Kyushu**: Singu and Tsuyasaki, Fukuoka Pref., on littoral rocks, I. UMEZAKI 1967, 1984 a, 20 and 21 May 1956 (IU) ; Ushinohama, Kagoshima Pref., on littoral rocks, I. UMEZAKI 2044, 2048 a, 25 May 1956 (IU) ; Tomioka, Amakusa island, on littoral rocks, I. UMEZAKI 2007 a, 2024, 23 and 24 May 1956 (IU). **Awaji island** : Yura, on littoral rocks, I. UMEZAKI 1450 a, 16 Jun. 1954 (IU). **Shikoku** : Cape Muroto, Kochi Pref., on littoral rocks, I. UMEZAKI 1457, 1466, 18 Jun. 1954 (IU) ; Cape Ashizuri, Kochi Pref., on littoral rocks, I. UMEZAKI 1473 a, 1493, 19 and 20 Jun. 1954 (IU) **Seto Inland Sea** : Ozuchijima island, Okayama Pref., on littoral rocks, I. UMEZAKI 1900, 4 Aug. 1955 (IU); in *Codium divaricatum*, I. UMEZAKI 1528, 1530, 23 Apr. 1954 (IU), in *Codium fragile*, I. UMEZAKI 1529, 23 Apr. 1954 (IU), I. UMEZAKI 1949 a, 4 Aug. 1955 (IU); Honjima island, Kagawa Pref., on littoral rocks, I. UMEZAKI 1923 a, 1924, 1948 b, 5 Aug. 1955 (IU). **Kii Peninsula** : Shirahama, Wakayama Pref., on littoral rocks, I. UMEZAKI 370, 21 May 1950 (IU), I. UMEZAKI 655, 656, 9 May 1952 (IU), I. UMEZAKI 676, 22 May 1952 (IU), I. UMEZAKI 754 a, 755 a, 9 Jun. 1952 (IU), I. UMEZAKI 765, 24 Jun. 1952 (IU), I. UMEZAKI 767, 18 Jul. 1952 (IU). **Shima Peninsula**: Wagu, on littoral rocks, I. UMEZAKI 608 a, 12 Mar. 1952 (IU), 708 a, 714, 9 Jun. 1952 (IU); Oshima island, on littoral rocks, I. UMEZAKI 625, 12 Mar. 1952 (IU), I. UMEZAKI 726, 731, 10 Jun. 1952 (IU). **Aichi Pref.** : Toyohama, on littoral rocks, K. OKAMUKA, Jul. 1902 (as *Brachytrichia Quoyi* in OKAMURA, Alg. Jap. Exsicc. no. 100, L, SAP) ; Himagajima island, on littoral rocks, K. INAGAKI, 21 Jul. 1948, I. UMEZAKI 343 b (IU). **Matsushima Bay** : Katsurashima island, on littoral rocks, I. UMEZAKI 1156 b, 1169 a, 8 Jul. 1953 (IU). **Amino**: Amino Bay, Kyoto Pref., on littoral rocks, I. UMEZAKI 1280 d, 31 Aug. 1953 (IU), in *Codium fragile*, I. UMEZAKI 959 b, 24 Feb. 1953 (IU). **Wakasa Bay** : Kato, Obama Bay, on littoral rocks, I. UMEZAKI 20, 29 Jun. 1949 (IU), I. UMEZAKI 56, 8 Sept. 1949 (IU), I. UMEZAKI 174, 9 Jul. 1950 (IU), I. UMEZAKI 194, 7 Aug. 1950 (IU), I. UMEZAKI 430 a, 21 Jun. 1951 (IU), I. UMEZAKI 581, 2 Mar. 1952 (IU), I. UMEZAKI 1892, 31 Jul. 1955 (IU), in *Codium fragile*, I. UMEZAKI 934 b, 31 Dec. 1952 (IU), I. UMEZAKI 969 b, 28 Feb. 1953 (IU), I. UMEZAKI 974 b, 15 Mar. 1953 (IU), I. UMEZAKI 1111 b, 29 Apr. 1953 (IU) ; Tsunekami, on littoral rocks, I. UMEZAKI 484 a, 485, 8 Aug. 1951 (IU) ; Takahama, on littoral rocks, I. UMEZAKI 352, 31 Jan. 1951 (IU) ; Koiso, Maizuru Bay, on littoral rocks, I. UMEZAKI 214 b, 18 Aug. 1950 (IU). **Hokkaido** : Muroran, on littoral rocks, I. UMEZAKI 1388, 21 Jul. 1947 (IU).

East Indies : on littoral rocks, Sarawak, Borneo, ZANARDINI (Type of *Brachytrichia rivularioides* ZANARD., L) ; Celebes, Fausses-Pisangs, Waru, Dammer and Roma, A. WEBER-VAN BOSSE, 1888–1889 (A. WEBER-VAN BOSSE, Siboga-Expedition, L).

Southeast Asia : on stones in the sea shore between tide marks, Dan, Koh Chang. Gulf of Siam, J. SCHMIDT, 10 Jan. 1900 (as *Brachytrichia Quoyi* in WITTR., NORDST., LAGERH., Algae exsicc. no. 1607, L).

Australia : on rocks in the upper littoral belt, west side, Middle River, Kangaroo island, South Australia, H. B. S. WOMERSLEY, 21 Jan. 1948 (as *Brachytrichia quoyi* in WOMERSLEY, Southern Australian marine algae, L) .

Indian Ocean : on littoral rocks, Ceylon, W. FERGUSON (as *Brachytrichia Quoyi* in W.

FERGUSON, Ceylon Algae no. 114, L) ; on littoral rocks, Ceylon, W. FERGUSON (as *Brachytrichia rivularioides* in Herb. G. THUR., L).

Altantic coast of North America : on *Fucus*, Woods Hole, Massachusetts, W. G. FARLOW (as *Hormactis Farlowii* in FARLOW, ANDERSON & EATON, Algae exsicc. Amer. Bor. no. 45, L) ; on *Fucus*, Woods Hole, Massachusetts, F. S. COLLINS, Jul. 1892 (as *Brachytrichia Quoyi* in HAUCK & RICHTER, Phyk. univ. no. 681, L) ; on *Fucus*, Woods Hole, Massachusetts, W. G. FARLOW (as *Brachytrichia Quoyi* in Herb. THURET, PC) ; on *Fucus*, Woods Hole, Massachusetts, W. A. SETCHELL 219, 236, 5 and 14 Aug. 1890 (as *Brachytrichia Quoyi* in W. A. SETCHELL, Algae of Massachusetts, L, D) ; on *Fucus*, Woods Hole, Massachusetts, W. A. SETCHELL 504, 18 Aug. 1892 (D) ; on *Fucus vesiculosus*, Quamquisset Harbor, near Falmouth, Massachusetts, W. A. SETCHELL, 1 Aug. 1890 (as *Brachytrichia Quoyi* in COLL., HOLD. & SETCH., Phyc. Bor.-Amer. no. 8, L) ; on *Fucus vesiculosus*, Quamquisset Harbor, near Falmouth, Massachusetts, W. A. SETCHELL, 5 Aug. 1891 (as *Brachytrichia Quoyi* in WITTR. & NORDST., Alg. exsicc. no. 1197, L) ; on *Fucus*, Falmouth, Massachusetts, F. S. COLLINS (as *Hormactis Quoyi* in COLLINS, Alg. Bor.-Amer., L) ; on dead *Fucus*, Quamquisset, near Falmouth, Massachusetts, R. A. HARPER, 18 Aug. 1891 (D) ; on breakwater in front of Ocean Terrace Hotel, Wrightsville Beach, North Carolina, HAROLD J. HUMM, 24 May 1946 (TSA, D) ; in *Codium dichotomum*, on beach near Fort Macon, Bogue Bank, Carteret County, North Carolina, H. L. BLOMQUIST, 30 Jul. 1951 (D).

Pacific coast of North America : on littoral rocks, Laguna Beach, California, N. L. GARDNER 2520, Nov. 1912 (Type of *Brachytrichia affinis* SETCH. & GARDN., UC) ; in pools at Arch Beach, east of Laguna, Orange County, California, N. L. GARDNER, Nov. 1912 (as *Brachytrichia Quoyi* in COLL., HOLD. & SETCH., Phyc. Bor.-Amer. no. 2106, SAP).

West Indies : on littoral rocks, Trisor Bay, Martinique island, Windward Islands, M. G. HAMEL & Mme A. HAMEL-JOUKOV, Apr. 1930 (as *Brachytrichia Quoyi* in Algues des antilles Francaises no. 4, L) ; on roots of *Rhizophora*, Cayo Maria Langa, Bay of Guayanilla, Puerto Rico, M. A. HOWE, 25 Jun. 1915 (D) ; on Mangrove trees, Culebra island, Puerto Rico, M. A. HOWE, 5 Mar. 1906 (as *Brachytrichia Quoyi* in North Amer. Mar. Alg. no. 4284, L) ; on aerial roots of black mangrove trees, Toxas Key, Bimini Bay, Bahama, H. J. HUMM, 30 Mar. 1948 (TSA).

Kyrtuthrix

ERCEGOVIĆ, Arch. f. Protistenk. 66 : 170, 1929. -Type species : *K. dalmatica* ERCEG.

Stratum irregulariter vel late expansum, frondibus unistratosis, planis, crustaceis, tenuibus, firmis ; filis parallelis, compactis, saepe medio affixis et utrinque rectis, saepe ramosis ; ramis V-formante ramosis ; vaginis firmis, hyalinis vel ex parte lutescentibus ; trichomatibus intra vaginam singulis, ad apicem in pilo attenuatis ; heterocystis intercalaribus ; multiplicatis hormogoniis et rarius sporis.

One species :

Kyrtuthrix maculans (GOMONT), comb. nov.

Brachytrichia maculans GOMONT in J. SCHMIDT, Bot. Tidsskrift 24 : 210, 1901. -Type from Koh Chang, Gulf of Siam, Siam (PC).

Kyrtuthrix dalmatica ERCEGOVIĆ, Arch. f. Protistenk. 66 : 170, 1929. *Brachytrichia dalmatica* FRÉMY, Mem. Soc. Nat. Sc. Nat. Math. Cherbourg 41 : 162, 1934.-Syntype, designated

here as the type, from Split, Jugoslavia (IU).

Frondes luteo-virides, in siccatate nigrae, filis 10–20μ crassis, 100–400μ, rarius 500μ altis ; trichomatibus 4–9μ crassis, ad apicem in pilo attenuatis ; articulis irregularibus, quadratis, cylindricis vel doliiformibus, 3–10μ longis ; heterocystis subquadratis, inferne longioribus, 6–11μ crassis, 4.5–15.5μ longis ; hormogoniis intra vaginam singulis, 17–35μ longis ; sporis sphaericis, 1.5–3μ crassis.

Habitat : Growing on rocks and rarely on large plants, or rarely perforating calcareous rocks, in the littoral belt or in the supralittoral belt, in tropical, subtropical and temperate marine waters.

Specimens examined :

Siam : on littoral rocks, Koh Chang, Gulf of Siam, J. SCHMIDT (Type of *Brachytrichia maculans* GOM., PC). **Viet Nam** : on rocks at a highest vegetation level, Nha Trang, E. Y. DAWSON 11254, 6 Feb. 1952 (D). **Indonesia** : on littoral rocks, Sumbawa, Solor, Fausses-Pisangs and Ceram, A. WEBER-VAN BOSSE, 1888-1889 (A. WEBER-VAN BOSSE, Siboga-Expedition, L).

Hawaii : on littoral rocks, Poipu Beach, Kausi, Hawaiian island. W. J. NEWHOUSE 53–101, 10 Sept. 1953 (D).

Japan : **Amami-oshima island.** Asani, on littoral rocks, I. UMEZAKI 1697, 6 Apr. 1955 (IU) ; Yamma, on littoral rocks, I. UMEZAKI 1740, 8 Apr. 1955 (IU) ; Gusuku, on littoral rocks, I. UMEZAKI 1719, 7 Apr. 1955 (IU) ; Koniya, on littoral rocks, I. UMEZAKI 1774, 1797 b, 9 Apr. 1955 (IU) ; Naze, on littoral rocks, I. UMEZAKI 1854, 12 Apr. 1955 (IU) ; Akakina, on littoral rocks, I. UMEZAKI 1864 a, 1867 a, 13 Apr. 1955 (IU). **Kyushu** : Kaimonsaki, Kagoshima Pref., on littoral rocks, I. UMEZAKI 2065, 26 May 1956 (IU) : Tomioka, Amakusa island, on littoral rocks, I. UMEZAKI 2001, 2005, 2015, 2019, 22–24 May 1956 (IU) ; Singu, Fukuoka Pret., on rocks in the supralittoral belt, I. UMEZAKI 1973, 20 May 1956 (IU). **Shikoku** : Shin-usa-machi, Kochi Pref., on littoral rocks, I. UMEZAKI 1428, 15 Jun. 1954 (IU) ; Cape Muroto, on littoral rocks, I. UMEZAKI 1453, 18 Jun. 1954 (IU) ; Cape Ashizuri, Kochi Pref., on littoral rocks, I. UMEZAKI 1479, 1492, 19–20 Jun. 1954 (IU). **Shimonoseki** : Yoshimi, on littoral rocks, I. UMEZAKI 248, 5 Nov. 1950 (IU). **Seto Inland Sea** : Ozuchijima island, Okayama Pref., on littoral rocks, I. UMEZAKI 1509, 23 Jun. 1954 (IU) ; Honjima island, Kagawa Pref., on littoral rocks, I. UMEZAKI 1665, 8 Feb. 1955 (IU). **Kii Peninsula** : Shirahama, Wakayama, Pref., on rocks in the littoral or supralittoral belts, I. UMEZAKI 382, 22 May 1951 (IU), I. UMEZAKI 504, 516, 31 Oct. 1951 (IU), I. UMEZAKI 518, 535, 540, 541, 1 Nov. 1951 (IU), I. UMEZAKI 642, 29 Oct. 1952 (IU) ; Hatakejima island, Tanabe Bay, on littoral rocks, I. UMEZAKI 444, 2 Aug. 1951 (IU) ; Kata, Kaiso-gun, Wakayama Pref., on littoral rocks, I. UMEZAKI 634 a, 27 Mar. 1952 (IU). **Shima Peninsula** : Oshima island, on litoral rocks, I. UMEZAKI 624, 12 Mar. 1952 (IU), I. UMEZAKI 724 a, 10 Jun. 1952 (IU), I. UMEZAKI 803, 804, 5 Oct. 1952 (IU) ; Wagu, on littoral rocks, I. UMEZAKI 694, 9 Jun. 1952 (IU), I. UMEZAKI 802, 4 Oct. 1952 (IU) ; Goza, on littoral rocks, I. UMEZAKI 592, 597, 11 Mar. 1952 (IU) ; Koshika, on littoral rocks, I. UMEZAKI 603, 11 Mar. 1952 (IU). **Izu Peninsula** : Hamazaki-mura, Kamo-gun, on littoral rocks, I. UMEZAKI 835, 8 Oct. 1952 (IU) ; Nagatsuro near Cape Iro, on littoral rocks, I. UMEZAKI 885, 891, 10 Oct. 1952 (IU). **Miura Peninsula** : Jyogashima island, Misaki-machi, on rocks in the supralittoral belt, I. UMEZAKI 1045 a, 30 Mar, 1953 (IU). **Boso Peninsula** : Kominato, on littoral rocks, I. UMEZAKI 1067, 3 Apr. 1953 (IU) ; Futomi, on littoral rocks, I. UMEZAKI 1092, 1094, 4 Apr. 1953 (IU). **Matsushima Bay** : Katsurashima island, on littoral rocks, I. UMEZAKI 1162 c, 8 Jul. 1953 (IU). **Amino** : Amino Bay, Kyoto Pref., on littoral

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Jugoslavia: perforating calcareous rocks, Split, Dalmatia, A. ERCEGOVIĆ, 16 Apr. 1957 (Syntype, here designated as the type, of *Kyrtuthrix dalmatica* ERCEG., IU), A. ERCEGOVIĆ, 20 Mar. 1957 (IU).

The type specimen of *Kyrtuthrix dalmatica* ERCEG., which was destroyed during last war, has not been available for this study, but I studied a syntype (here designated as the type) of the species which was sent by Dr. ERCEGOVIĆ who collected it in Split, Dalmatia, on April 16, 1957. The specimen is preserved in my herbarium.

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On the Extractability of Fish Muscle Proteins*

By

Yutaka SHIMIZU and Wataru SIMIDU**

Introduction

It is generally accepted that the properties, such as color, stiffness, texture, flavor, gel-forming ability, etc., of fish meats are mainly originated from the proteins contained in their muscles. The fact that these properties are widely different for different fishes and for their post-mortem history makes it difficult to control the manufacturing process of various fish meat products. Especially, in the manufacture of "kamboko", a fish meat sausage produced specifically in this country, the materials are limited to only several fishes due to their gel-forming ability appropriate to this product.

DYER[1] found that the change in flavor and texture in frozen fish meats was mainly due to the decrease of soluble actomyosin during the cold storage. MIYAKE and HAYASHI[2] showed that the amount of actomyosin extracted from fish muscles using DYER's method was dependent upon the kind of fish.

In view of these facts, studies on the proteins which may be related with the properties of fish meat should be of considerable significance and, especially, detailed information about the extraction of those proteins is of importance as the first step of such studies.

Various kinds and various concentrations of salt have been used to extract muscle proteins ; 0.6 M KCl contained 0.04 M $NaHCO_3$ and 0.01 M Na_2CO_3 by WEBER and MEYER (1933)[3], 10 per cent NaCl by REAY (1933)[4], 7 per cent LiCl by SMITH (1934)[5], 0.5 M KCl contained 0.03 M Na_2CO_3 by GREENSTEIN and EDSALL (1940)[6] and 5 per cent NaCl contained 0.02 M $NaHCO_3$ by DYER, FRENCH and SNOW (1950)[7]. Among these extractants***, those specifically prepared for the fish muscle are REAY's solution for haddock and DYER's for cod.

The components of protein to be extracted may depend on the kind and concentration of the extractants used. We were puzzled to establish which extractant is most effective for fish muscle proteins. The experiments reported in this paper were undertaken to obtain information about the necessary condition for the complate extraction of soluble proteins and actomyosin from the fish muscle using the method of solubility determination and the viscometry.

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** Laboratory of Food Chemistry

***Basides these salt solutions, a rather weak sodium hydroxide solution has also been used for the turbot muscle by HIRANO (1952)[8].

Methods

1. Extraction Method

In order to extract the constituent proteins from the muscle, both mechanical destruction of the muscle cells and effective extractant solution are required. To the former a waring blender or a homogenizer specially designed for the muscle is most effective and convenient of many other procedures, e. g., grinding the muscle with sand in a centrifuge tube or the freeze-slicing technique.

When the homogenizer is used with a rather strong salt solution, a froth formation accompanied with protein denaturation may occur, which results in a very low recovery of the soluble protein. DYER et al[7] inserted a plastic plate in the blender jar to prevent the sample solution from direct contact with air.

In the present study, another method was used to avoid the surface denaturation of proteins due to foaming. The fish muscle freed from melandrya was chopped by a knife or a chopper. A given volume of the chopped meat was homogenized using a blender (made by *Nihon Seiki Kogyo Co., Ltd.*) for about 2 minutes in the presence of 1.5 or 2 volumes of distilled water chilled to 0°C. No froth formation was observed during this operation. The paste-like suspension obtained was immediately transferred into 20 volumes of an extractant solution. After a desired period of extraction, the solution was centrifuged for 20 minutes at 3,500 to 4,000 r. p. m. to remove the stroma and the non-peptizing proteins. The degree of extraction was determined by measuring the protein nitrogen in an aliquot of the supernatant.

2. Preparation of Actomyosin Precipitate.

10 ml. of the NaCl extract was diluted to 0.08 M with ice cold water and the flocculent precipitate then formed was allowed to settle down overnight at 0°C. The actomyosin precipitate thus obtained was centrifuged from the solution.

3. Determination of Proteins.

The total nitrogen in the muscle was determined by the Kjeldahl method and the soluble protein nitrogen in the extract by the biuret method modified by MATSUMOTO and KANAMITSU[9]. Non-protein nitrogen was determined by measuring nitrogen in an aliquot of the filtrate after the protein had been precipitated at a concentration of 7 per cent of trichlor acetic acid.

4. Measurement of Viscosity.

A Brookfield type rotational viscometer (*Tokyo Keiki Seisakusho Co., Ltd.*) was used for the measurement of the viscosity of the fish muscle extracts. Seven rotors of different dimensions were selectively used according to the magnitude of the viscosity of given solutions. The readings taken after steady state had been attained, since the viscosity decreased in the initial stage of rotation. All the measurements were carried out at 3-5°C. with an accuracy at±0.3°C at a given temperature.

The sample solutions used for the viscometry were prepared by homogenizing 60

g. of lizzard fish muscle with 420 ml. of extractant in the same manner described above for the extraction method and then by filtering off the stroma with a cotton cloth.

Fig. 1a shows the viscosity η (in centipoises) of the salt extracts of lizzard fish muscle plotted against the apparent velocity gradient D, where D tentatively presents the number of rotation of the rotor per unit minute. All the curves in this figure indicate a typical non-Newtonian flow and, as shown in as shown in Fig. 1b, follow satisfactorily the viscosity law of de WAELE-OSTWALD[10]:

$$\eta = KD^n \qquad (1)$$

where η is the viscosity, D is the velocity gradient and K and n are constants.

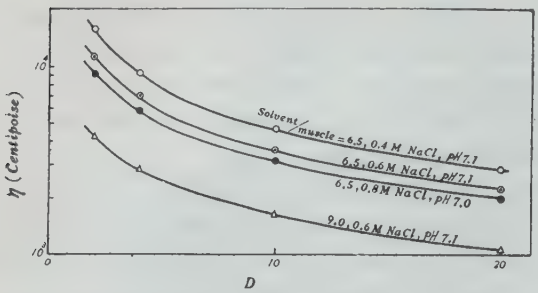

Fig. 1a. Relation between viscosity of muscle extract and velocity gradient: D represents number of rotation of roter per unit minute.

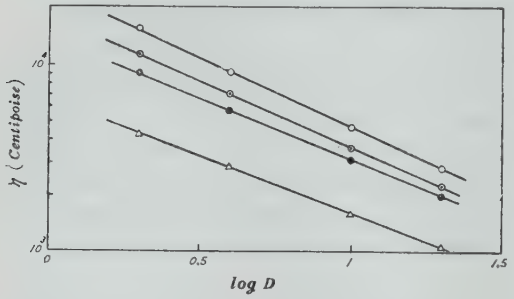

Fig. 1b. Relation between log η and log D

Results and Discussion

1. Extraction Period

The effect of extraction time on the extractability of soluble proteins and actomyosin was investigated on several kinds of fish muscle. These experiments were made using as extractants 20 volumes of water, of 0.6 M NaCl, and of 1.5 M NaCl, all buffered with $NaHCO_3$ to pH 6.8-7.2.

Fig. 2 shows the changes in extractability of the soluble proteins and the actomyosin of striped bass muscle in these extracts as a function of extraction time. It is found that the extractability of salt soluble proteins increased considerably with the extraction period and attained to a maximum after 2 or 3 hours, and that these increases were mainly due to that of actomyosin. The fact that longer the extraction period yielded a larger amount of actomyosin had been found by KITABAYASHI[11] for

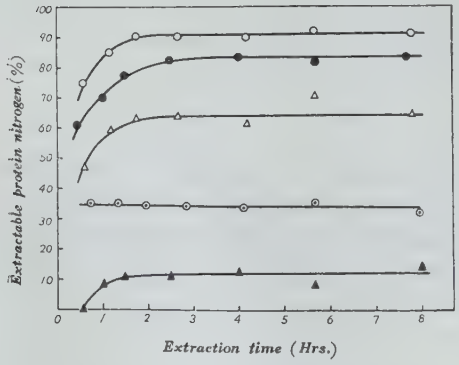

Fig. 2. Eflect of extraction period on the extractability of soluble proteins and actomyosin fraction in striped bass muscle; ◉ soluble proteins in water, ○ soluble proteins in 0.6 M NaCl, ● soluble proteins in 1.5 M NaCl; △ actomyosin fraction in 0.6 M NaCl, ▲ actomyosin fraction in 1.5 M NaCl.

turbot muscle. It was observed in the course of such increase of extracted protein that a marked thixotropy accompanied with an intense turbidity appeared in 0.6 M extract but not in 1.5 M extract. In contrast to the salt extractable proteins, the water soluble proteins were much readily extracted, and this indicates that their properties were entirely different from those of salt soluble proteins.

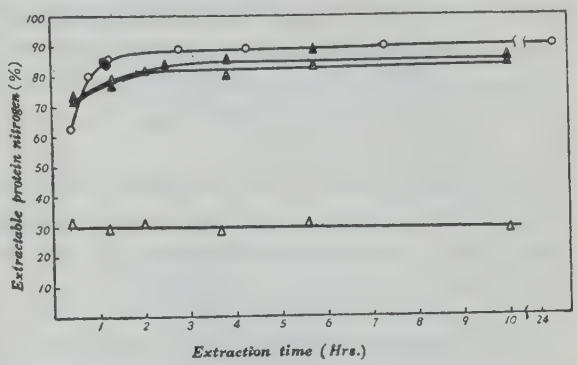

Fig. 3. Effect of extraction period on the extractability of soluble proteins in chub mackerel and lizzard fish muscle. △ water soluble, ◬ 0.6 MNaCl soluble, ▲ 1.5 M NaCl soluble proteins for chub macke-rel ; ○ 0.6 M NaCl soluble proteins for lizzard fish.

It is worthy of noticing that the amount of the actomyosin precipitated from 1.5 M extract was so small as no more than one-fifth the total soluble protein, while that from 0.6 M extract amounted to 70 per cent of the total. This may be attributed either to the depolymerization of F-actin or to the dissociation of actomyosin in such a strong salt solution as 1.5 M NaCl.

A similar relation between extraction period and extractability of proteins was also obtained for mackerel and lizzard fish muscles (Fig. 3).

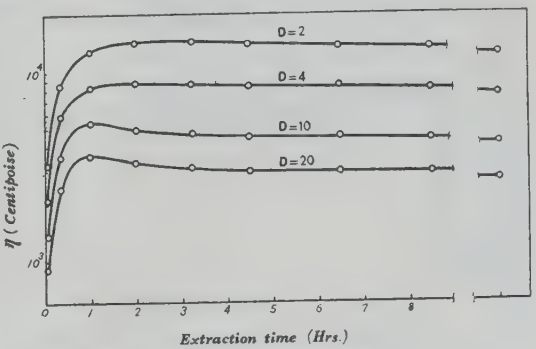

Fig. 4a. Effect of extraction period on the viscosity of 0.6 M NaCl extract.

Next, the viscosity of 0.6 M extract of a lizzard fish muscle buffered with 0.015 M $NaHCO_3$ to pH 7.0 was investigated. The results are shown in Fig. 4a, where each curve corresponds to a particular "apparent" velocity gradient of flow indicated. The viscosity increases markedly in the initial stage of extraction and maintains its value or decreases slightly after two hours of extraction, depending on the velocity gradient used. In Fig. 4b are plotted the values of index n in the de WAELE-OSTWALD viscosity equation (1) as a function of extraction time. The values of n have been determined by plotting the data of Figure 4a at various fixed extraction times on a log η vs. log D graph and then evaluating the

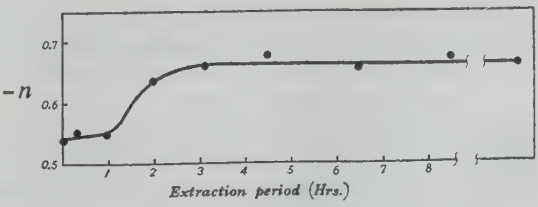

Fig. 4b. Change of non-Newtonian behavior in 0.6 M NaCl extract as a function of extraction period ; n denotes the index in de Waele-Ostwald's viscosity equation.

slopes of the resulting straight lines. Since the index n is a measure of the non-Newtonian behaviour of flow, Fig. 4b suggests that some change may occur in the configurations of proteins extracted between one and two hours from the start of extraction ; as well-known, configurations of long chain molecules are responsible for the non-Newtonian behaviour of solutions of such molecules. Referring to the previous investigations[12] on the interactions of myosin and action, this marked change in n between one and two hours of extraction may be ascribed to an actomyosin formation from myosin and F-action.

From these results, it is concluded that complete extraction of actomyosin as well as soluble proteins from fish muscles requires about 2 hours. This agrees well with 1.5 to 2 hours recommended by EDSALL[18] for the rabbit muscle.

2. NaCl Concentration of Extracting Medium

The effects of concentration of NaCl and pH on the extractability of soluble proteins and actomyosin had been investigated by DYER et al. They adopted a 5 per cent NaCl as the extractant and succeeded in extracting 90 to 92 per cent of protein from cod. In the present study, the extractability of proteins contained in rigor muscles of sriped bass, lizzard fish and mackerel in various concentrations of NaCl was investigated. The pH values of these extracts were controlled to 6.6–7.0 with phosphate buffer (ionic strength $\frac{\Gamma}{2}=0.02$). The degree of extraction was determined by measuring the protein nitrogen in solution after 3 hours extraction.

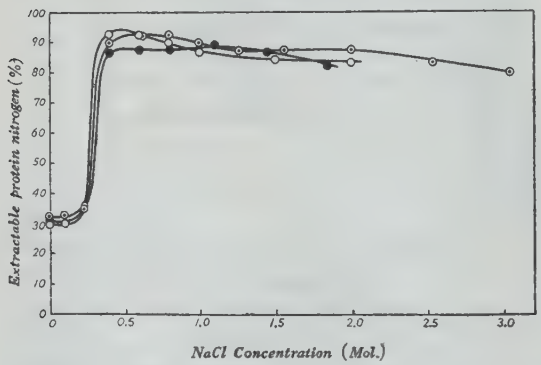

Fig. 5. Effect of concentration of NaCl on the extractability of soluble proteins from ◉ lizzard fish muscle, ○ striped bass muscle, and ● chub mackerel muscle.

The results are shown in Fig. 5. There is a remarkable increase in solubility between 0.2 and 0.4 M ; such concentrations have been accepted as the salting-in threshold of actomyosin[14]. Maximum extraction is obtained between 0.4 and 1.0 M NaCl where 88 to 93 per cent of total proteins are extracted, according to the kind of fish muscle. Such a feature in the solubility curve was commonly observed in all the fishes used in this experiment and also in the cod used by DYER et al. It is of interest that turbid extracts exhibiting a thixotropy were obtained between 0.4 and 1.0 M NaCl and semitransparent nonviscous solutions at 1.5 and 2.0 M NaCl. These facts may indicate that the concentrations between 0.4 and 1.0 M make actomyosin formation easy.

Fig. 6 shows effects of NaCl concentration upon the viscosity of the lizzard fish muscle extracts buffered their pH values to 6.8–7.0. The viscosity remarkably increases between 0.2 and 0.4 M, attains a maximum between 0.4 and 0.6 M, and beyond this range shows a gradual decrease followed by a rapid drop. These results may indicate

that the molecules of large dimensions such as actomyosin more predominated in the weak salt extracts than in the strong salt extracts. This consideration was confirmed by examining the amount of actomyosin precipitated by dilution of the extracts (Fig. 9); between 0.4 and 0.6 M a maximum amount of precipitate was obtained, being equivalent to 70 per cent of the total soluble protein, while at 0.8 and 1.5 M only 65 and 23 per sent, respectively. Such a decreasing tendency of actomyosin with increasing salt concentration of extract can also be observed in DYER's results on cod, though in their data the observed decrease is slower than that in the present experiment.

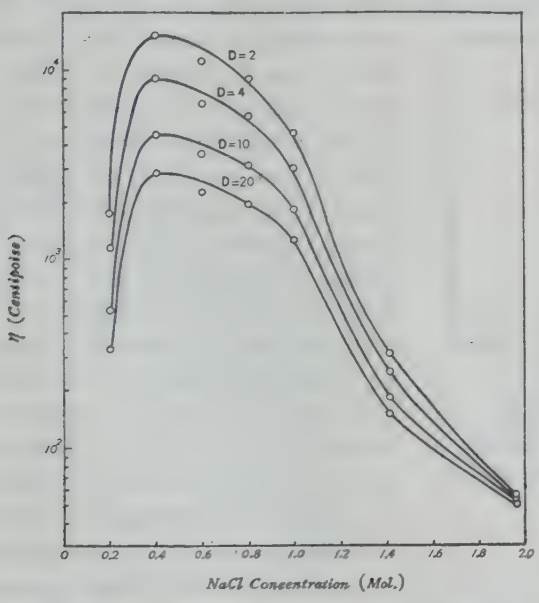

Fig. 6. Viscosity of muscle extract obtained from various concentrations of NaCl.

It appears from these results that the formation of actomyosin from F-actin and myosin is markedly influenced by salt concentration of extractant, being promoted in 0.4 to 0.6 M NaCl and retarded in 1.5 M or above. Thus it is suggested that NaCl concentrations of 0.4 to 0.6 M be most adequate for the complete extraction of soluble proteins containing actomyosin from the fresh muscle; DYER's solution (ionic strength $\frac{\Gamma}{2}=0.85$) might be fairly too strong for the purpose concerned.

3. *Condition of Muscle*

It is well-known that protein denaturation gives rise to some decrease in its solubility, and therefore the extraction of muscle proteins from fishes must be carried out with as fresh a sample as prossible. Use of this fact makes it possible to evaluate the degree of deterioration of quality in stored fish. REAY[5] previously found that the protein fraction which had become insoluble in the frozen haddock muscle was the globulin fraction, which he obtained by dilution and acidification of the 10 per cent NaCl extract. In recent studies the state of muscle in frozen[15)16)],

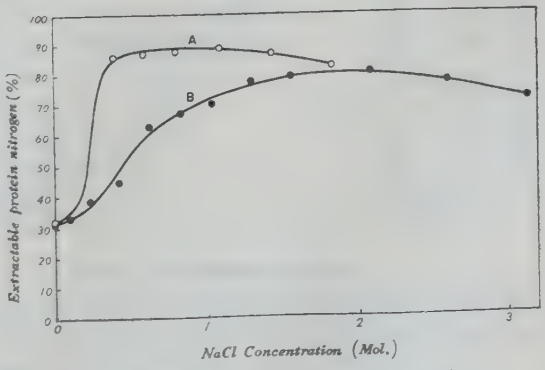

Fig. 7. Influence of cold storage (for 7 days at 2-4°C) on the extractability of soluble proteins for chub mackerel muscle; ○ rigor muscle. ● stored muscle.

salted[17] or dried fishes[18] has been discussed in connection with the decrease of the actomyosin due to these processings.

In this experiment, the solubility curves in various concentrations of NaCl obtained using cold stored fish muscles were compared with those for fresh muscles. Figs. 7, 8 and 9 show the solubility curves of the muscle proteins in striped bass, mackerel and lizzard fish, all of which were stored for 7 days at 2 to 4°C after the rigor-mortis stage. Comparison of curve A and B shows that remarkable change may take place in the weak salt soluble fraction. The rapid rising of extractability ovserved in curve A between 0.2 and 0.4 M turns to a gradual increase between 0.2 and 1.0 M in curve B. The extractabilities of water soluble and strong salt (above 1.5 M) soluble proteins are practically kept constant even after 7 days storage. Such a remarkable decrease of soluble protein between 0.4 and 1.0 M after the cold storage may be due to the change of actomyosin fraction, as observed from Fig. 9. This result is in agreement with those obtained in the above-mentioned investigations for other processings.

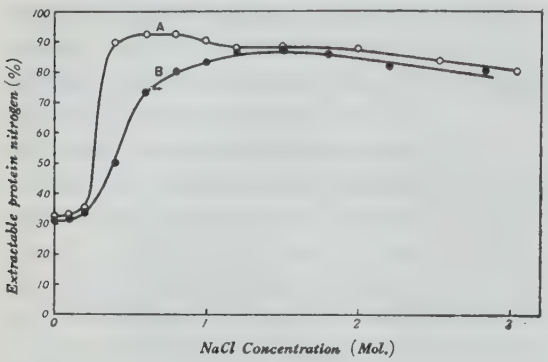

Fig. 8. Influence of cold storage (for 7 days at 2–4°C) on the extractability of soluble protiens for lizzard fish muscle; ○ rigor muscle. ● stored muscle.

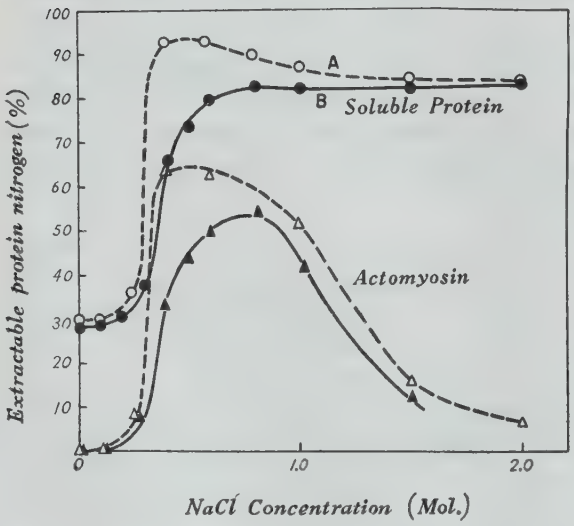

Fig. 9. Influence of cold storage (for 7 days at 2–4°C) on the extractability of soluble proteins and actomyosin fraction in striped bass muscle. ○ soluble proteins, △ actomyosin fraction for rigor muscle; ● soluble proteins, ▲ actomyosin fraction for stored muscle.

Based on these experimental findings it is recommended that 0.4 to 0.6 M NaCl extractants be most effective to estimate the deterioration in the quality of fish; it is believed that the estimation of the freshness of fish muscle in terms of the extraction method will be greatly facilitated by the fact that marked decrease in extractability takes place in this NaCl concentration range.

4. *pH of Extracting Medium*

DYER et al investigated the effects of pH on protein extraction using a 5 per cent NaCl solution, and found that the maximum extraction was obtained between pH 7 and 9 and a very rapid decrease between pH 5 and 6.

It has been mentioned above that the component of extractable protein were quite different in accordance with the NaCl concentrations of extractant used; those extractants were water, 0.6–1.0 M and 1.5–2.0 M NaCl. The effects of pH on the extraction of protein were investigated in these different concentrations of extractant. The given extractants were brought to desired pH with phosphate buffer or acetate buffer $\left(\frac{\Gamma}{2}=0.02\right)$, and the final pH was measured using a glass electrode pH meter. The results are shown in Fig. 10.

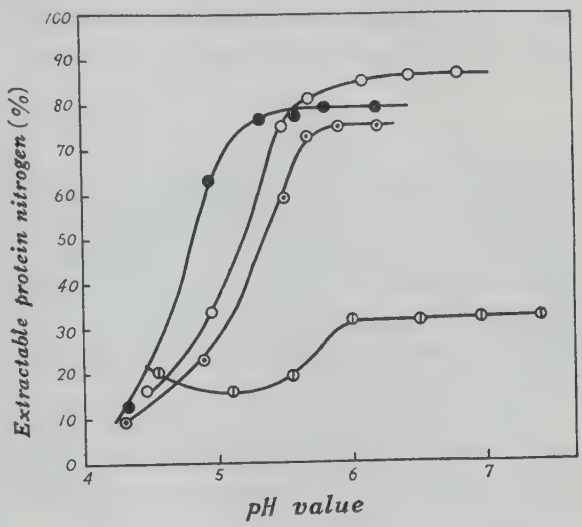

Fig. 10. Effect of pH on the extraction of soluble proteins at various fixed concetrations of NaCl. ⓘ water, ○ 0.6 M NaCl, ● 1.5 M NaCl, ◉ 3.0 M NaCl.

In the four curves shown, it is found that the pH has influence on the extractability of proteins in various extents, depending on NaCl concentration of extractant; the pH ranges in which a rapid increase of extraction appears are 6.0–5.5, 5.5–5.0, 5.0–4.5 and 5.5–5.0 for concentrations of 0, 0.6, 1.5 and 3.0 M, respectively. Thixotropy of the extracted solution was observed only in the 0.6 M extracts adjusted their pH to 5.7 or above. It will be seen that the curve for 0.6 M extract is shifted to a slightly acidic side compared to that for the 5 per cent salt extract reported by DYER et al on cod. The reason of this difference is unknown to us, but the long extraction period (3 hours) used in our experiment may be responsible for it.

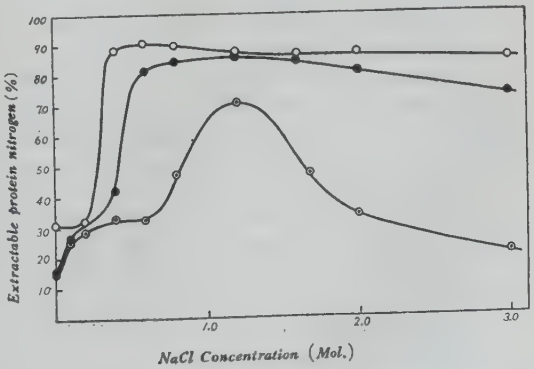

Fig. 11. Effect of pH on the solubility curve in various concentrations of NaCl. ○ pH 6.8–7.0, ● pH 5.3–5.6, ◉ pH 4.8–5.1.

Fig. 11 plots the solubility against concentration of NaCl at fixed pH values. As

may be expected from the results in Fig. 10, the extraction at moderate concentrations of salt (1.0–1.5 M) is not so much affected by pH values within the range studied here. On the other hand, those at weak salt concentrations (0.4–0.6 M) and also at strong salt concentrations (above 2 M) are influenced strongly by pH values. These facts may be ascribed to the lack of actomyosin in the former and to the salting-out effect on the non-actomyosin proteins in the latter.

Summary

Necessary conditions for the complete extraction of soluble proteins as well as actomyosin fraction from the fish muscle were investigated by using NaCl solution as the extractant. The optimum salt concentration of the extracting medium ranged from 0.4 to 0.6 M NaCl at pH 6.5 to 7.0. The optimum extraction period was 2 or 3 hours. Under such conditions more than 90 per cent of the total proteins were extractable from fresh muscles and about 70 per cent of those were precipitated by dilution in the form of actomyosin.

A remarkable contrast was noticed between the week salt extracts (0.4–1.0 M NaCl) and the strong salt extracts (above 1.5 M) for the viscosity and the thixotropy, and this was responsible for the actomyosin formation being affected with the ionic strength of the extracting medium.

The decrease in protein extractability occurred in cold stored fishes was attributed to that of actomyosin fraction, in agreement with DYER's observation on frozen cod.

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Bacteriostatic Actions of Some Antiseptics on Commercial Potato Starch Contaminating Bacteria and their Effects on the Thermal Death Rate of those Bacteria

By

Saburo UENO and Wataru SIMIDU*

In the course of manufacturing preservative seasoned foods, it is preferable to use as low a temperature as possible for heat processing in order to keep their original textures and flavors. In general, however, bacterial deterioration is unavoidable for foods heat processed at temperatures below 100°C. It is expected that foods with good preservativity may be obtained even with a low temperature heat processing if suitable antiseptics are used in conjuction with appropriate packing of the products. In previous studies[1,2] we observed on spores of some aerobic bacilli that rates of thermal death of the spores were enhanced and that the germination of survived spores was retarded by the presence of antiseptics. This paper reports on a similar study undertaken to confirm these observations for bacteria contaminating commercial potato starch used in the manufacture of fish-sausage and similar food stuffs.

Experimental

1. Thermal death rate of bacteria.

One gram of a high grade potato starch (produced in Hokkaido) suspended in 100 ml. of sterile physiological saline in a conical flask of 300 ml. capacity, and the suspension obtained was incubated at 38°C., with occasional shaking of the flask. At definite intervals a given volume of the suspension was taken out, and part of it was used for the counting of bacteria grown and the remaining part was consumed for the measurement of the thermal death rate. The number of bacteria was determined using the ordinary dilution method, where the bacteria was plated on a common nutrient agar (pH 7.0) as usual and cultivated at 38°C. for 48 hours. The number of colonies developed was taken as the number

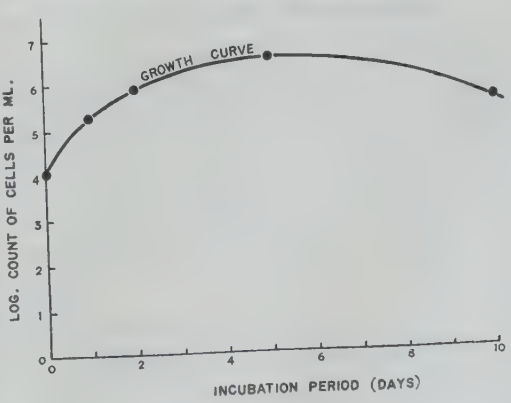

Fig. 1. Growth curve of protato starch contaminating bacteria when starch was suspended in a physiological saline and incubated at 38°C.

* Laboratory of Food Chemistry

of bacteria to be counted. The growth curve obtained in this way is shown in Fig. 1.

The determination of the thermal death rate was made according to the following procedure. The bacteria suspension described above was diluted with sterile physiological saline, accompanied with vigorous stirring to ensure uniformity of the solution. Then it was poured into a number of test tubes (18 x 180 mm), with 2 ml. for each. After these tubes had been heated in a water bath at a temperature either of 88±0.3°C. and 90±0.3°C. for various intervals of time, the test tubes were taken out of the bath and cooled immediately in running water to 20°C. These temperatures of heating were selected because preliminary experiments had indicated that sterilization effects of antiseptics concerned were most tractable to study in this temperature region. The content of the tube was poured on a common nutrient agar medium of pH 7.0 and plate-cultured at 38°C. for 48 hours. Although, in general, the germination of heat survived bacteria is retarded due to their heat dormancy, the period of recovery culture of 48 hours used in this work porved satisfactory to estimate the thermal death rates of the bacteria studied.

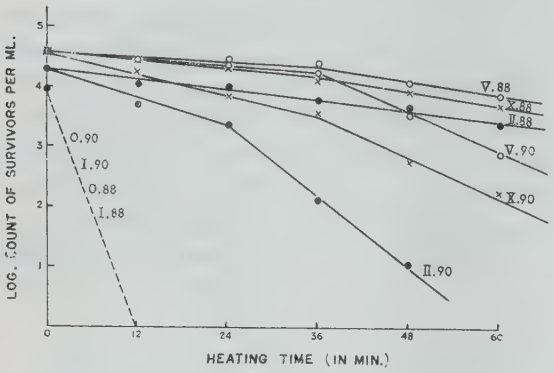

Fig. 2. Thermal death rate of potato starch contaminating bacteria when starch was suspended in physiological saline and incubated at 38°C. for a given time.
0, I, II, V, X, incubation period (day)
Numbers attached, heating temperatures (°C.)

The number of survived spores was determined from the number of colonies developed, and is plotted against time of heat-treatment in Fig 2. The "decimal reduction time (D. R. T.)" and the "mortality time (M. T.)" are defined in accordance with those initiated by

Incubation period (day)	Heating temperature (°C.)	Decimal reduction time (in min.)		Mortality time (in min.)
		Initial stage	Later stage	
0,I	90	12>	12>	12>
0,I	88	12>	12>	12>
II	90	26	10	32
V	90	82	21	68
X	90	37	18	54
II	88	72	66	120
V	88	126	64	135
X	88	82	65	150

Table 1. Thermal death rate of potato starch contaminating bacteria heated in the physiological saline with change in the incubation period of the organisms.

KATZIN[3] et al. and by HELEN[4]. The decimal reduction time means the time required for heating until the number of bacteria at a given heating time decreases to one tenth the initial value; and the mortality time means the time required for heating until the number of bacteria decreases to one-hundredth the value for nonheattreatment. The D. R. T. values have been determined from both slopes of the initial stage (above knick) and of the later stage (below the knick) of the death rate curves shown in Fig. 2. The values obtained for these two slopes are listed in Table 1, designate "initial stage" and "later stage", respectively. The M. T. values have read off the curves in Fig. 2, and are recorded in Table 1.

Microscopic observations and heat shock tests of the bacteria suspensions showed that the population of the bacteria suspension consisted almost of non spores in the early stage of incubation, and was gradually predominated by the bacterial spores on prolonged incubation, reaching a maximum number on the fifth day. Subsequently, the population decreased with increasing incubation period. Fig. 2 shows that the thermal resistance of the bacteria has a maximum value at a time close to the 5 days' incubation at either of heating tempreatures of 88°C. and 90°C. This indicates that the bacteria have the highest heat resistance after some period of maturation in the given medium. The decrease in heat resistance of the bacteria on prolonged incubation is due probably to culture products. In testing the bacteria incubated for 5 days and 10 days, the cell density was adjusted as equal as possible with each other in order to eliminate an error originated from the difference in cell density. Such an error was pointed out by YESAIR[5], ESTY[6] and others.

2. Effect of antiseptics upon thermal death rate.

Starch suspension were made using entirely the same procedure as described above and incubated at 38°C. for four days; this condition of incubation was chosen because the experiments above had indicated that maximum heat resistance was obtained under this condition. The suspensions were then filtered under reduced pressure using a Buchner funnel with a coarse filter paper (Toyo filter paper No. 5A). The filtrate was centrifuged at room temperature. The precipitated cells were resuspended in sterile physiological saline, followed by recentrifugation. This procedure was repeated four times and then the cells were thoroughly washed. Finally, the cells were suspended in an appropriate volume of sterile physiological saline and again filtered through a Toyo filter paper No. 5A to remove clumps. The filtrate so obtained was stored in an ice box at 0°C., and was supplied for experiments.

This stock cell suspension, physiological saline, and a solution of a test antiseptic dissolved in sterile physiological saline were mixed in such a way that the concentration of antiseptic became equal to 50 p.p.m. and that the number of bacteria in the mixture became approximately to a desired value. With the mixture so prepared the thermal death rate measurements were carried out as described above.*

* Preliminary experiments indicated that antiseptics introduced from the cell suspensions into the recovery media amounted to less than 0.2 p.p.m., so that its effect might be neglected.

The modes of thermal death of the bacteria at a temperature either of 88°C. and 90°C. are shown in Fig. 3 and Table 2. The values of D.R.T. and M.T. listed in Table 2 have been obtained from the death rate curves in Fig 3, using the same method as described in Experiment 1. Evaluation of the sterilization effects of antiseptics in terms of the M.T. shows that the antiseptics cause the decreases of M.T. ranging from about 18% to 53% at 88°C and from 14% to 58% at 90°C., respectively, in comparison with the control containing no antiseptic. It is seem from the figure and table that thermal death rates of spores are markedly increased by addition of antiseptics and, that when comparing at equal concentrations, this increase is most pronounced for vitamin K_3, followed by Furaskin, and penta chlorphenol in order. The sterilization effects generally decreased when the heating temperature was sufficiently high, in agreement with MURRAY.[7]

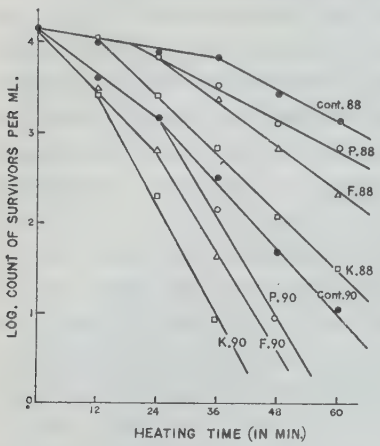

Fig. 3. Thermal death rate of resting cells of potato starch contaminating bacteria heated in physiological salines with and without various kinds of antiseptic at 88°C. and 90°C.
F.……5-nitro-2-furfuraldehyde semicarbazone (Furaskin)
P.……penta chlorphenol
K.…… 2-methyl-1.4-naphthoquinone (vitamin K_3)
Cont.… Control (without antiseptic)
Numbers attached, heating temperatures (°C).

Relations between the logarithm of number of survived bacteria and the time of heating are represented by two intersecting straight lines, in agreement with the finding of our previous investigations[8~14].

3. Effects of antiseptics upon the germination of spores survived from heat treatment.

After the cell suspension used in Experiment 2 had been heated at 88°C. for either 30 or 60

Table 2. Thermal death rate of resting cells of potato starch contaminating bacteria heated in physiological saline with and without various kinds of antiseptic at 88°C. and 90°C.

Antiseptic		Heating temperature (°C.)	Decimal reduction time (in min.)		Mortality time (in min.)
Kind	Concentration (P. P. M.)		Initial stage	Later stage	
Furaskin	50	88	62	25	64
Pentachlor phenol	50	88	91	35	84
Vitamin K_3	50	88	50	20	48
None (control)		88	91	42	102
Furaskin	50	90	25	10	33
Pentachlor phenol	50	90	31	11	36
Vitamin K_3	50	90	25	9	26
None (control)		90	31	14	42

minutes, they were cultivated at 38°C. for 24 hours and 48 hours on common nutrient agar media of pH 7.0 containing 50, 15 and 5 p.p.m. of a given antiseptic, respectively. The number of colonies developed was counted, and its ratio to the corresponding number obtained on the culture medium without antiseptic was defined as the germination rate. Similar experiments were conducted also for non-heat treated spores chosen as a control.

Antiseptic		Cultivated period (in hr.)	Germination rate (%)		
			Heating time (in min.)		
Kind	Concentration (p.p.m.)		0	30	60
Furaskin	50	24	23	0	0
		48	27	0	0
	15	24	68	0	0
		48	80	0	0
	5	24	100	1	0
		48	100	6	1
Pentachlor phenol	50	24	13	0	0
		48	18	0	0
	15	24	55	0	0
		48	100	60	40
	5	24	100	100	100
		48	100	100	100
Vitamin K_3	50	24	0	0	0
		48	0	0	0
	15	24	15	5	0
		48	27	10	0
	5	24	50	60	8
		48	100	100	12
none (control)		24	100	100	100
		48	100	100	100

Table 3. Germination rates of spores of potato starch contaminating bacteria subjected to lethal heat treatment (at 88°C.) and then cultivated on common nutrient agar media of pH 7.0 containing various kinds of antiseptic.

The results obtained are shown in Table 3. The germination of the spores survived from heat treatment is markedly suppressed by antiseptics compared to those not heat treated prior to cultivation. For heat treated spores this action is strongest with Furaskin, followed by vitamin K_3, penta chlorphenol in order. For non-heat treated spores vitamin K_3 shows the strongest action and the actions of the other two are practically the same.

Conclusion

The results obtained in this work with commercial potato starch contaminating

bacteria show features entirely similar to those reported previously on *Bacillus mesentericus* and *Bacillus mycoides*. Therefore, it may be concluded that the patterns of action of antiseptics on various kinds of spore are esssentially not different with each other. It is also suggested from the present results that addition of suitable antiseptics makes it possible to prepare preservative foods without resort to unfavorable high temperature heat processing.

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General Theory of Fishermen's Current Measuring Device, *Siomi-ito*

By

Tasae KAWAKAMI, Kaoru MITSUHASHI and Otohiko SUZUKI*

In Japanese commercial purse seine fisheries, a simple current measuring device called *siomi-ito* is in traditional use in coastal fishing areas, where there is some possibility of occurrence of predominant underwater current, in order to avoid the entangling of net which occurs not seldom in actual fishing operation. In Japanese, *sio* means literally tidal current, *mi* means to observe or to detect, and *ito* means a fine string. It is a simple expedient of dropping overboard several boulder sinkers attached to the end of strings of different lengths into the water. Each sinker is subjected to the current of the corresponding depth according to the length of string. The tilt of the string at the surface of the sea may be determined by both profile of the current velocity and length of the string. By a glance of tilt angles of the string at the surface, an experienced fisherman can infer the violent underwater current whether it is unfavourable for seining operation or not. The judgment is said to be hardly possible only by experienced fishermen : They judge erroneously at times and the crew suffers from trouble to get out of the entangling of net. Fishermen, therefore, are in a great care in manipulating their *simoi-ito*.

It would be a great manipulative advantage in any fishery to know in advance the current at fishing depth. The importance of the underwater current for pelagic fishery such as purse seining or trawling has been emphasized by Dr. J. N. CARRUTHERS[1,2], who devised a simple current measuring instrument called "current cone". The current pattern of the coastal region is very complicated and changes unexpectedly. It is quite unsafe to infer the strength and direction of underwater current from the visible surface water movement. For fishery use, it is therefore demanded that the current at each depth can be detected successively without loss of time, even if it is a rough estimation. If the *siomi-ito* has an accuracy of certain degree, it seems to answer these difficult requirements.

In the days when the modern ultrasonic fish-locater was not yet in common use, *siomi-ito* was used to detect also schools of fish below water surface. Fishermen could detect a school by means of the faint feelings produced by the contact of fish body to the string. Thus it has been also known as "*atari*" or "*ateyoma*", which means feeling or feeling line. The make up of a *siomi-ito* has depended largely on the preference of the individual concerned, so that we can not find the general mode of construction for it.

In a preceding paper[3], one of the writers has discussed the mechanical theory of *siomi-ito* in common use in the present fishery and have proposed a proper way of its

*Laboratory for Fishing Gear Research

arrangement and its manipulation. In what follows, an attempt is made to establish a general thory of *siomi-ito* and propose some new designs.

Mechanical Analysis

When a spherical sinker is suspended in a given stationary horizontal current by means of a flexible string of diameter D, it is desired to know the angle θ_0 which the string makes with the vertical at the surface of water. In order to make the problem more general, suppose that the current profile is arbitrary, namely the velocity U is given as an arbitrary function of the depth z.

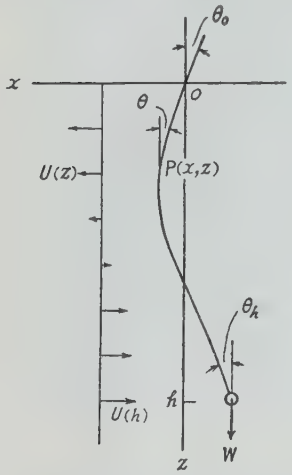

Fig. 1

Now it is assumed (1) that the weight of the string is compensated by its buoyancy, (2) that the force acting on the string per unit length is normal to the string and its magnitude varies proportionally with the square of the sine of the angle between the string and the stream, and (3) that the frictional force which acts tangentially to the string can be neglected as compared with the normal component.

Figure 1 shows a string carrying a spherical sinker in a current. Choose the origin, O, of a rectangular coordinates, (x, z), at the water surface; the string passes through the origin. The x-direction is taken as the direction of current and the z-direction is taken positive downward. Let s be the arc length of string measured from the origin to any point P(x, z) and θ be the angle which the string makes with the vertical direction. Then the equilibrium of the string element ds requires

$$-T\frac{d\theta}{ds}=k[U(z)]^2\cos^2\theta, \tag{1}$$

where T is the tension of the string, which is constant if the frictional force be negligible, $U(z)$ is the current velocity at the depth z, and k is a proportionality constant depending on the diameter, D, of the string as follows:

$$k=\frac{1}{2}C_D\rho D\equiv aD, \tag{2}$$

C_D being the drag coefficient of the string when it is normal to the stream and ρ the density of water. To obtain the vertical coordinate of the form of the string, we have

$$dz=\cos\theta ds. \tag{3}$$

The integration of these differential equations from the surface, $z=0$, to a depth $z=h$, with the condition that $\theta=\theta_0$ for $s=0$, gives

$$\frac{k}{T}\int_0^h U^2dz=\log\tan\left(\frac{\pi}{4}-\frac{\theta_0}{2}\right)-\log\tan\left(\frac{\pi}{4}-\frac{\theta}{2}\right).$$

For problems in which the angle θ is in the range less than 20°, a negligible error is

introduced by the substitution that

$$\cos\theta = 1, \quad ds = dz, \quad s = z,$$

$$\log\tan\left(\frac{\pi}{4} - \frac{\theta}{2}\right) = \theta.$$

Thus the expression can be simplified to

$$\frac{k}{T}\int_0^h U^2 dz - \frac{k}{T}\int_0^h U^2 ds = \theta_0 - \theta.$$

At the end of the string of length h, is attached a spherical sinker whose apparent weight in water is W and whose radius is R. The angle of inclination, θ_h, of the string at this end when it is subjected to a current of velocity $U(h)$ is, therefore, represented by

$$\tan\theta_h = \frac{K[U(h)]^2}{W},$$

where

$$K = \frac{1}{2} C'_D \rho \pi R^2 \equiv b R^2, \tag{4}$$

C'_D being the drag coefficient of the sphere. If the angle be small this can also be simplified to

$$\theta_h = \frac{K[U(h)]^2}{T}.$$

Hence we obtain

$$\frac{k}{T}\int_0^h U^2 ds = \theta_0 - \frac{K[U(h)]^2}{T}. \tag{6}$$

This is the most general expression which gives the relation between the velocity of current and the tilt angle of the string at the surface when the angle is small.

In order to measure the current velocity at a depth h, the following types of measurement may be available:

(1) Different length type, and

(2) Different sinker type.

Different Length Type

Siomi-ito's commonly used in commercial fisheries belong to this type and its mechanical properties have been discussed by one of the present writers in his previous paper.[3] Two strings, one of which is longer than the other by length L but both of which carry similar sinkers of the same weight and same dimensions, are suspended in water in such a way that the sinker of the short string reaches the depth $z=h$, and hence the lower end of the long string may locate at the depth $z=h+L$. The whole arrangement is shown schematically in Figure 2. Let $\theta_{z=h}$ and $\theta_{z=h}$

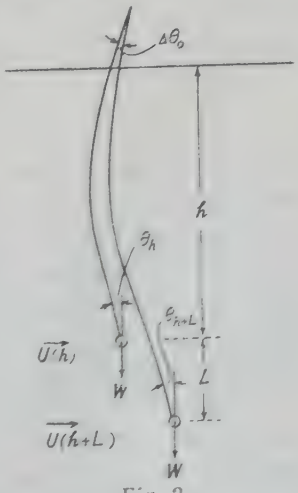

Fig. 2.

be the surface tilt angles of short and long strings respectively, and $\Delta\theta_0$ be the difference of these tilt angles, namely, $\Delta\theta_0=\theta_{0,2}-\theta_{0,1}$. Then for the short string, we have

$$\frac{k}{T}\int_0^h U^2ds=\theta_{0,1}-\frac{K[U(h)]^2}{T}, \tag{7}$$

and for the long string

$$\frac{k}{T}\int_0^{h+L} U^2ds=\theta_{0,2}-\frac{K[U(h+L)]^2}{T}. \tag{8}$$

On subtracting we obtain

$$\frac{k}{T}\int_h^{h+L} U^2ds=\Delta\theta_0-\frac{[U(h+L)]^2-[U(h)]^2}{T}K.$$

If L be so short that the velocity gradient in this region can be considered negligibly small, we have approximately

$$\int_h^{h+L} U^2ds=\frac{[U(h)]^2+[U(h+L)]^2}{2}L. \tag{9}$$

Hence we obtain

$$\frac{1}{T}\left(\frac{kL}{2}+K\right)[U(h+L)]^2-\frac{1}{T}\left(\frac{kL}{2}-K\right)[U(h)]^2=\Delta\theta_0. \tag{10}$$

This equation may be used to determine the values of $U(h+L)$ from the known values of $U(h)$ and $\Delta\theta_0$. If current measurements are required simultaneously at several depths, we must use at once several strings, of which the lengths s are differed by length L in succession; thus the current velocities at an interval L can be estimated layer by layer from the surface toward the bottom by measuring the differences of tilt angles, $\Delta\theta_0$, between two consecutive strings.

In order to save the trouble in computation, some ideas on the design of *siomi-ito* are conceivable. Let us suppose the case in which

$$L=\frac{2K}{k}, \tag{11}$$

then the equation (10) is simplified to

$$\frac{kL}{T}[U(h+L)]^2=\Delta\theta_0. \tag{12}$$

The computation will then be greatly simplified, because the current velocity at the depth $(h+L)$ can be calculated from the measurement of the value of $\Delta\theta_0$ only. If L is so small that we can put $U(h)=U(h+L)$, the condition (11) may be excluded. In these equations, since the values of K and k are given by ordinary hydrodynamic theory as represented by the equations (2) and (4), the length L may be determined by the diameter, D, of the string and the radius, R, of the sinker, and T by the apparent weight of the sinker in water.

There occurs a new idea in this type. Consist the long string with two parts, namely, the main part which is similar to the short string both in length, h, and in diameter, D, and the additional part whose length and diameter are L and D' respectively. The additional part is introduced between the bottom end of the main string and the sinker. Then the equation (8) becomes

$$\frac{k}{T}\int_0^h U^2ds+\frac{k'}{T}\int_h^{h+L}U^2ds=\theta_{0,2}-\frac{K[U(h+L)]^2}{T},$$

where k' is the value of k for the additional part, corresponding to D' instead of D. Thus the equations (10), (11) and (12) may hold by simply converting k into k'. It seems that, if the diameter of the main string be chosen as thinly as possible within the limits of breaking strength, and if the diameter of the added string be so thick, consequently the length L be so short that the current in the layer of observation is considered to be uniform, we would be able to minimize the influences due to the disturbances occurred in the intermediate layer, and the measurement would become much accurate. This arrangement would be effective for deep water measurement. In what follows, this type of *siomi-ito* is named the "different string type".

Different Sinker Type

If the velocity of current varies discontinuously or nonlinearly with the depth, for example as is the case with skin current of the sea surface, the approximation represented by the equation (9) does not hold well. In this case the following method which may be called the "different sinker type" would be useful.

This type of *siomi-ito* consists of two strings of the same length, and of the sinkers of different sizes, denoting their radii by R_1 and R_2 respectively, but of the same apparent weight, T, in water ; thus the large sinker, $R=R_1$, may be subjected to a larger hydrodynamic force than the small sinker, $R=R_2$, even if both of them are placed in an identical current. When these two sinkers are suspended in a layer of depth h, in which the velocity is $U(h)$, the application of the equation (6) gives

$$\frac{k}{T}\int_0^h U^2ds=\theta_{0,i}-\frac{bR_i^2[U(h)]^2}{T}, \qquad (13),\ (14)$$

where $i=1$, 2, and b is a constant given by the equation (4). Subtracting the equation (14) from (13) we obtain

$$\theta_{0,1}-\theta_{0,2}\equiv\Delta\theta_0=\frac{b[U(h)]^2}{T}(R_1^2-R_2^2),$$

or numerically

$$[U(h)]^2=\frac{1.43T\Delta\theta_0}{(R_1^2-R_2^2)}, \qquad (15)$$

by which the current speed at the depth h may be calculated from the measurement of $\Delta\theta_0$.

The large sinker is not always only one, but two or more separate sinkers of the same total weight may serve the same purpose. In this case, if n sinkers of the same size $R=R_1$ are used in place of a single sinker, the equation (15) becomes

$$[U(h)]^2=\frac{1.43T\Delta\theta_0}{(nR_1^2-R_2^2)}. \qquad (16)$$

Thus the sensitivity can be improved.

It may be noteworthy that the subtraction of the equations for the both strings

signifies the elimination of the disturbances in the intermediate layer. This may hold well if the current varies two dimentionally in a horizontal plane. It is clear that the direction of current is inferred from the long string or the string carrying the large sinker relative to the short string or the string carrying the small sinker.

Test Experiments

A series of trial tests in the open sea was made with a *siomi-ito* of the different length type designed as follows : The sinker was made of a hollow brass sphere in such a way that it could be separated into two hemispherical bowls in which a lead ballast was charged. The size of the sinker was 3.8 cm. in radius ; this size made the actual operation handy. Its apparent weight in water was adjusted by changing the weight of the ballast. In this test two kinds of weight were used to adapt the instrument for measurements over a wide speed range, viz.,

Weight (1), for low speed use : When the sinker was charged with this weight, it weighed 250 g. in water,

Weight (2), for high speed use : When the sinker was charged with this weight, it weighed 1,000 g. in water.

As regards the string, if it be a twine, roughness of its surface should be smoothed by coating with some material such as plastic film, to avoid the yawing action[4] which may arise by the helicoidal pattern of the strands. In this test two kinds of string were used, viz.,

String (1), Hemp twine coated with plasticized polyvinyl chloride $D=2.5$ mm.,

String (2), Nylon filament for angling use, $D=0.45$ mm.

The length difference, L, of the strings is given by the equation (11), in which the numerical values are $a=0.55$, $b=0.71$ in c.g.s. unit. Therefore,

$$L=\frac{2bR^2}{aD}=\frac{37.5}{D},$$

thus we have the following numerical values :

for String (1), $L=1.5$ m.,

for String (2), $L=8.3$ m.

In order to avoid the troubles in computation represented by the equation (12), a convenient scale for reading the current speed was made in such a way that the value of U cm./sec. could be read directly from the scale by measuring the distance, x cm., between two points, one to each string, at 1 m. below the supporting point P as shown in Figure 3. In this case we have

$$U^2\frac{kL}{T}=\frac{x}{100}.$$

Hence, for the low speed sinker or Weight (1),

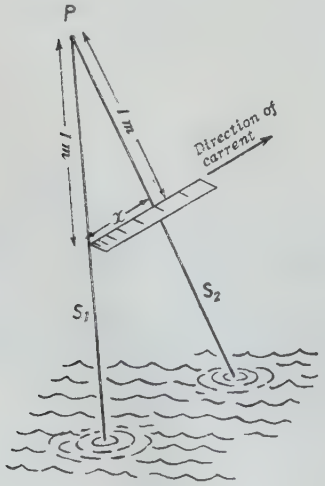

Fig. 3.

$$U^2 = 122\,x,$$

and for the high speed sinker or Weight (2),

$$U^2 = 486\,x.$$

An example of the scale is shown in Figure 4. The direction of the current is inferred from the point on the long string, S_2, relative to the point on the short string, S_1.

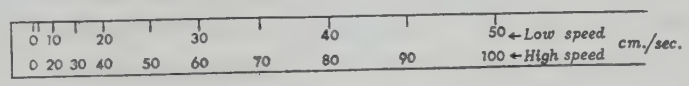

Fig. 4.

In July 1956, test experiments were conducted. The readings of the *siomi-ito* were compared with the values obtained by a standard current meter of the Ekman type. The results are summerized in Table 1.

Table 1.

Depth (m.)	Ekman current meter (cm./sec.)	*Siomi-ito* Reading (cm./sec.)	*Siomi-ito* Error (%)	20%-discount (cm./sec.)
20	16	19	19	15
30	26	30	24	24
2	15	18	20	14
2	25	37	48	30
2	12	14	11	11
2	29.8	*28.3		

* Different string type, made up with some correction in its design taking the former experiences into consideration.

It is seen from this table that the *siomi-ito* has a tendency of over-estimation, but the values obtained by 20%-discount show a good coincidence with values obtained by the Ekman meter, as shown in the last column of the table. This may be ascribed to the fact that the numerical values of the drag coefficients have not been adequate. The general tendency, however, seems to be promissing, since the correction of such a systematic error would be a simple matter.

Other series of trial tests for a *siomi-ito* of the different sinker type was made. The large sinker in this case consisted of two spherical sinkers, each of which was quite similar in its size to that used in the preceding test, but the total weight of these two sinkers was reduced to 243 g. The small sinker was a replete lead sphere of 243 g. in weight in water and 1.8 cm. in radius. Thus the numerical values appeared in the equation (16) were as follows:

$$T = 243 \times 980 \text{ dyne}, \quad n = 2, \quad R_1 = 38 \text{ cm.}, \quad R_2 = 1.8 \text{ cm.}$$

Then the formula representing the relation between the values of U and $\Delta\theta_0$ becomes

$$U^2=\frac{1.43\times2.38\times10^5}{2\times144.4-3.24}\Delta\theta_0=1.32\times10^2\Delta\theta_0.$$

Hence the current measuring scale for this case should be graduated by the equation:

$$U^2=132\,x.$$

In October 1957, test experiments were made in the Bay of Maizuru. In this test the experimental boat was drifted by wind over the sea and the relative velocity between the boat and water was measured by the *siomi-ito* together with a standard Ekman current meter. The results obtained are shown in Table 2.

Table 2.

Depth (m.)	Ekman current mater (cm./sec.)	*Siomi-ito* Reading (cm./sec.)	*Siomi-ito* Error (%)	15-%discount (cm./sec.)
2	32.0	35.3	9	30.0
2	22.3	27.7	24	23.5
2	33.3	37.9	14	32.2
2	29.8	34.3	15	29.2

It can be seen from this table that the *siomi-ito* of this type also has a tendency of over-estimation in some degree, but the values of 15%-discount show a fairly good agreement with the Ekman current meter.

Generally speaking, if it is intended to measure the current at large depth, the string inclines so largely that the error due to the approximation in the theory cannot be neglected. In such case, fine strings would be advantageous. Under the condition that the velocity U cm./sec. of the current is uniform, and the diameter of the string is 0.05 cm., the length H m. of the string of the large sinker to be veered out in order that the tilt angle of the string at the surface reaches 20° is tentatively computed as a function of U. The result is shown in Table 3. It can be seen that the *siomi-ito* of this type can be used for the depth of about 50 m. when the current speed is half knot.

Table 3.

U (cm./sec.)	H (m.)
20	78
30	30
40	13
50	6

It is mentioned in passing that the current velocity was found fluctuating considerably, not only in its magnitude but also in its direction, especially in the regions of complicated topography. In a special case, for example at a point in the Bay of Maizuru, the current turned its direction nearly 360° within a few minutes. Although this is an extreme case, the ordinary current meter would not be available in such cases.

A well designed *siomi-ito*, although it is a rough measuring device, would be an effective gear for fishing operation or for research in coastal regions on account of its handiness and its simplicity.

Acknowledgment

In addition to the thanks to the colleagues of the Kyoto Fisheries Experiment station who made generous assistances in carrying out the field experiments, the writers wish to record here that the expences for this study were partly defrayed by the research fund from the Japanese Ministry of Education.

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Studies on the Histamine Formation of *Proteus morganii*

By

Masao KIMATA and Akira KAWAI*

In the course of studies on the biochemistry of *Achromobacter histamineum*, KIMATA, one of the authors has shown that, caused by the growth of this organism, the rate of histamine formation in fish meat-extracts considerably varies with the kinds of fish supplied as culture media, and presumed the presence of some unknown substances which accelerate the bacterial activity of decarboxylating histidine in fish meat-extracts[1].

The present work has been undertaken in order to detect the possible substances that will stimulate the histamine formation of this organism. Although *Achromobacter histamineum* is a bacterium which we have succeeded in isolating and named as a new species, we have recently found that this species is nearly identical with *Proteus morganii* from the results of our detailed comparative studies[2]. Accordingly, the typical strain of this bacteria, *Proteus morganii* NCTC 235 has been used throughout the present work.

In this paper is described the effect of several kinds of chemical present in the culture media upon the yield of histamine attended with the bacterial growth.

Experimental

Method *Proteus morganii* NCTC 235 from the Laboratory's stock is grown in a common broth at 30°C. After being incubated for a day, the grown cells have been harvested, washed, resuspended in sterile saline, and then used as the organism to be introduced in the inoculum.

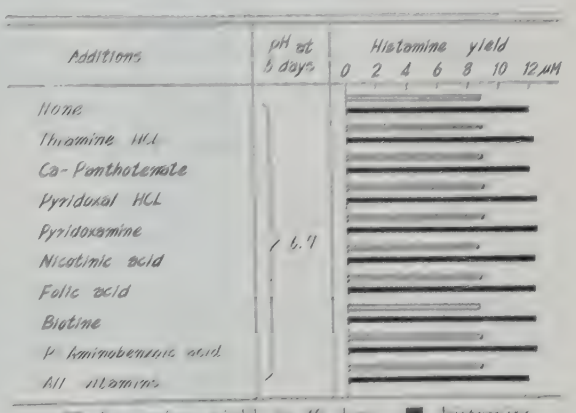
Two kinds of basal medium are used in this study, namely a fish meat-extract solution prepared from the yellow tail by the method already reported[3] (this solution is mixed with distilled water and L-histidine to give such concentrations of total nitrogen and histidine as 0.48 mg./ml. and 48.44μM./ml, respectively) and the chemically defined synthetic medium (50 mg. NaCl, 20 mg. KH_2PO_4, 2 mg. $MgSO_4 \cdot 7H_2O$, 20 mg. L-sodium glutaminate, 20 mg. L-asparagine, 20 mg. sodium acetate, 200γ calcium panthotenate**, 200γ nicotinic acid**, and 20μM. L-histidine are filled to 10 ml. with distilled water). The definite amount of the chemicals is added to each 5 ml. (or 2.5 ml. in some cases as noted in the Figs. attached) of these basal media in a test tube, and filled to 10 ml. with distilled water, and then sterilized by autoclaving as usual. Those chemicals including glucose, fructose, L-ascorbic acid and sodium thioglycollate are, however,

* Laboratory of Microbiology

** Lack of panthotenic acid and nicotinic acid scarcely supports the bacterial growth in the synthetic media, and these vitamins are supplemented.

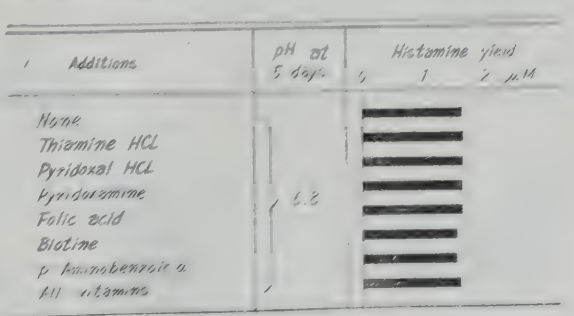
sterilized previously in their solutions and then mixed under aseptic condition. The initial pH of the meat-extract media is 6.0, that of the synthetic media being 6.4.

At a temperature of 30°C is the incubation done of the whole cultures inoculated. As a preliminary experiment has indicated that the production of histamine reaches to a state of equilibrium in 3 or 4 days, the amount of accumulated histamine in the cultures is determined after 5 days' incubation, the method already reported[6] being used. At the same time the pH of the culture media is also determind. Each set of the experiments is performed in duplicate, including a control without test chemicals.



□ histamine yield in 40 hrs. ■ histamine yield in 120 hrs. Vitamins added, 5γ/ml, respectively.

Fig. 1. The effect of vitamins upon histamine formation of *Proteus morganii* in the fish meat extract media.



Vitamins added, 5γ/ml, respectively.

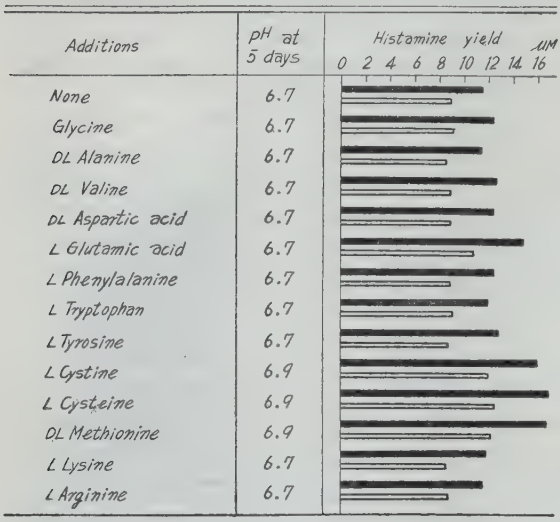
Fig. 2. The effect of vitamins upon histamine formation of *Proteus morganii* in the synthetic media.

Results Effect of vitamins. As for various kinds of vitamin B complex, the results obtained are illustrated is Figs. 1 and 2. From the Figs., we can see that none of the vitamins affect histamine formation to a significant degree. Neither pyridoxal nor pyridoxamine is effective. This accords with the several data already reported about the other bacteria, namely, a fact that histidine decarboxylases require no pyridoxal group as a co-enzyme[7], or if not so, the demand is at least not so great as in other amino acid decarboxylases[8].

Effect of amino acids. In the experiment by means of the meat-extract media, 13 kinds of amino acid have been tested, as shown in Fig. 3. As for the synthetic media, glutamic acid can not be tested because this chemical has already been presented in the basal media, as a necessary element for the bacteria to give sufficient crops. The Figs. 3 and 4 show that cystine, cysteine, methionine and glutamic acid are only effective of the amino acids in our experiments. The other chemicals appear to have little or no effect on histamine formation. The effect of cystine and cysteine seems unchanged regardless of their concentrations, although histamine formation seems somewhat stimulated in some of the concentration, as shown in Figs. 5 and 6.

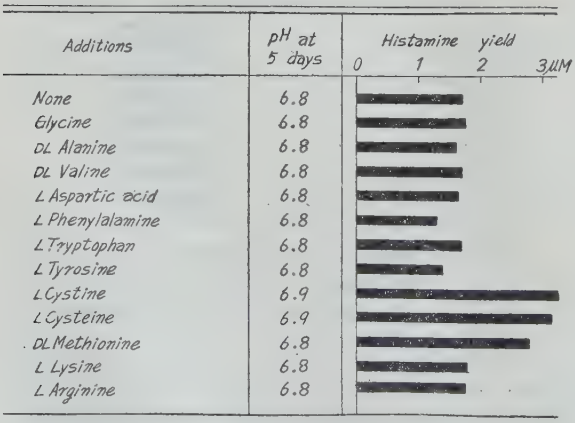
Effect of carbohydrates. As carbohydrate such as glucose has long been regarded

as important for amino acid decarboxylation, the authors have ascertained the rôle of these metabolites in the present bacterial action. Fig. 7 clearly indicates in our experiment that each of carbohydrates has increased the yield of histamine in the meat-extract media. Of these compounds, glucose and fructose are remarkably active. As seen in Fig. 8, histamine formation is on the increase as the concentration of glucose increases in density. On the contrary, in case of the synthetic media no similar phenomenon is found, that is, the amount of histamine to be formed remains almost unchanged by the glucose, as shown in Fig. 9.



□ histamine yield in 40 hrs. ■ histamine yield in 120 hrs. Amino acids added, 0.5μM./ml, respectively.

Fig. 3. The effect of amino acids upon histamine formation of *Proteus morganii* in the fish meat-extract media.



Amino acids added, 1μM./ml, respectively.

Fig. 4. The effect of amino acids upon histamine formation of *Proteus morganii* in the synthetic media.

Effect of cyst(e)ine and glucose in their combination. we have also discovered that histamine formation is remarkably increased when cyst(e)ine and glucose are added to the basal media at the same time. Results of our experiments concerning this finding are shown in Figs. 10～13. These Figs. clearly shown that in case of the meat-extract media the degree of stimulation is larger in the combined action of cyst(e)ine and glucose than in their separate one. But in the synthetic media glucose alone has no effect on the degree of stimulation. It it also evident from the Figs. that any concentration of glucose with the same amount of cystine has nothing to do with the stimulative power, except one which has a very little glucose, say 0.02% in the media, and that it remains almost unchanged by the amount of cystine in the same concentration of glucose.

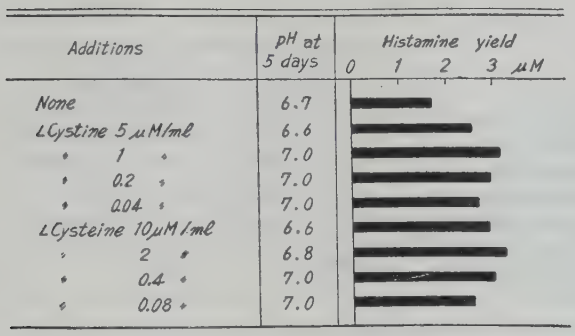


Fig. 5. The effect of cystine and cysteine in their various concentrations upon histamine formation of *Proteus morganii* in the fish meat-extract media.

2.5 ml. of the basal media are used as the media.

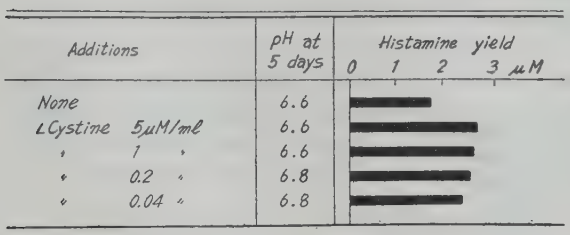


Fig. 6. The effect of cystine in its various concentrations upon histamine formation of *Proteus morganii* in the synthetic media.

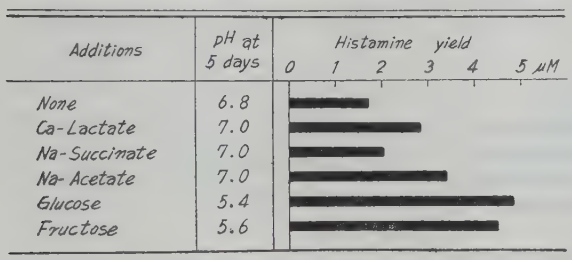


Fig. 7. The effect of carbohydrates upon histamine formation of *Proteus morganii* in the fish meat-extract media.

2.5 ml. of the basal media are used as the media.

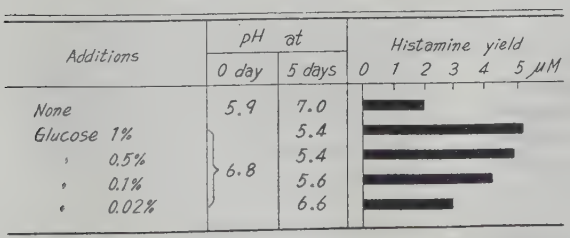


Fig. 8. The effect of glucose in its various concentrations upon histamine formation of *Proteus morganii* in the fish meat-extract media.

2.5 ml. of the basal media are used as the media.

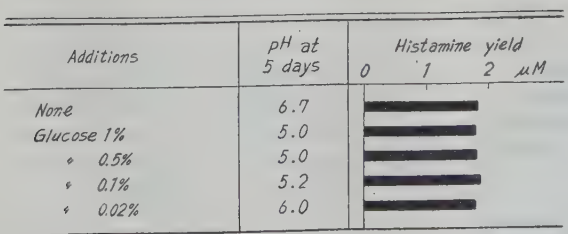


Fig. 9. The effect of glucose in its various concentrations upon histamine formation of *Proteus morganii* in the synthetic media.

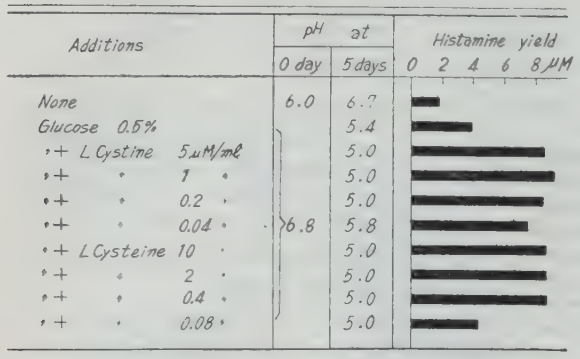

Additions
pH at
0 day
5 days
Histamine yield
0 2 4 6 8 μM
None 6.0 6.7
Glucose 0.5% 5.4
+ L Cysteine 5 μM/ml 5.0
+ " 1 " 5.0
+ " 0.2 " 5.0
+ " 0.04 " 6.8 5.8
+ L Cysteine 10 " 5.0
+ " 2 " 5.0
+ " 0.4 " 5.0
+ " 0.08 " 5.0

Fig. 10. The effect of cystine and cysteine in their various concentrations upon histamine formation of *Proteus morganii* in the fish meat-extract media supplemented with glucose.

2.5 ml. of the basal media are used as the media.

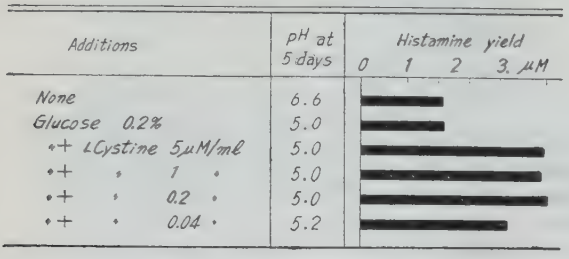


Fig. 11. The effect of cystine in its various concentrations upon histamine formation of *Proteus morganii* in the synthetic media supplemented with glucose.

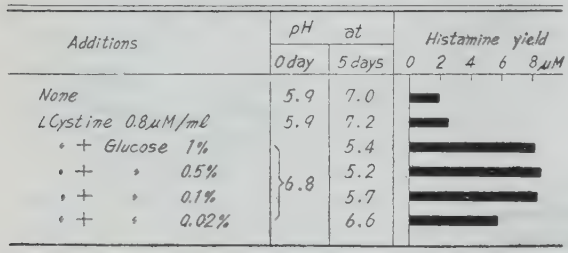


Fig. 12. The effect of glucose in its various concentrations upon histamine formation of *Proteus morganii* in the fish meat-extract media supplemented with cystine.

2.5 ml. of the basal media are used as the media.

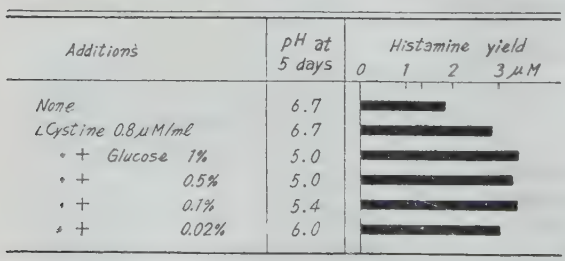


Fig. 13. The effect of glucose in its various concentrations upon histamine formation of *Proteus morganii* in the synthetic media supplemented with cystine.

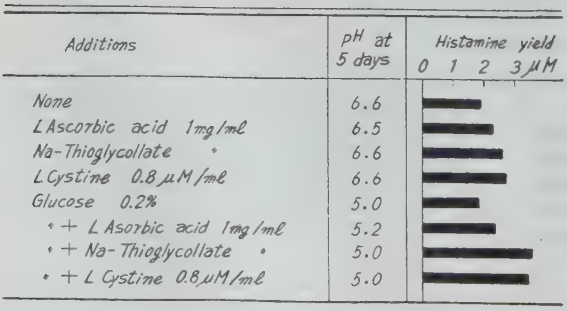


Fig. 14. The effect of redox potential-reducers and that of the combination of the same reducers and glucose upon the histamine formation of *Proteus morganii* in the synthetic media.

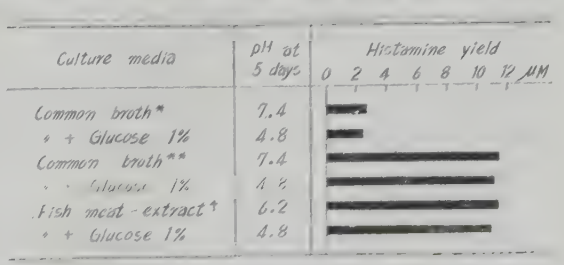
Discussion

In this work, we have obtained some interesting informations about the histamine formation caused by the growth of *Proteus morganii*, although our purpose which is stated at the beginning of this paper has not been pursued.

We have found that of the amino acids which we have tested cyst(e)ine and methionine have strong stimulating influence on histamine formation through the power of *Proteus morganii*. But we must not presume that any one of these chemicals will serve as a limiting factor in the biosynthesis of histidine decarboxylase. A similar thing is found in these amino acids that they can be metabolized by this organism, consequently liberating hydrogen sulphide[7] which may reduce the redox potential of the culture media. This consideration may be confirmed by the fact that cyst(e)ine or methionine can be replaced by other redox potential-reducers, such as ascorbic acid and sodium thioglycollate, as shown in Fig. 14. Therefore, it seems likely that the stimulating power of cyst(e)ine or methionine is due to its power of reducing the redox potential of the culture media.

Glutamic acid seem to be effective, though slightly, in the fish meat-extract media, and this amino acid is necessary for the growth of this organism in the synthetic media, as already mentioned. Thus, the rôle of glutamic acid in histamine formation remains to be seen.

Carbohydrates, such as glucose, have been found effective in the fish meat-extract media, but it is not so in the synthetic ones. For this the following explanation may be given. A growing bacterium generally requires a certain amount of carbohydrate as an energy source to support its metabolic function including the biosynthesis of various kinds of enzyme protein. There is a considerable difference in the amount of carbohydrate between the two kinds of medium employed in our work. So the basal media consisting of a diluted solution of fish meat-extract may have been deficient in the amount of carbohydrate which is essential in supporting histamine formation, and an addition of glucose to it must have considerably increased histamine in yield. This point of view may give an account of Arao's observation[7] that glucose promotes histamine formation of *Proteus morganii* i. e. as his basal medium consists of histidine, inorganic salts and 0.05 % of yeast extract, his media may have been as deficient in energy sources as the diluted solution of fish meat-extract we have used. Furthermore, this consideration



* Amount of histidine, 3.42μM./ml. ** Amount of histidine (enriched), 13.42μM./ml. † Amount of histidine, 13.42μM./ml.; total nitrogen, 1.48mg./ml.

Fig. 15. The effect of glucose upon histamine formation of *Proteus morganii* in several kinds of complex medium.

may be partly demonstrated by the fact that the stimulation caused by glucose can not be found in the media which are richer in energy sources as in Fig. 15.

It is also noteworthy that this organism decomposes glucose present in the media and consequently the reaction becomes acidic. Our previous work[2] has shown that the histidine decarboxylase of this organism is most active under pH about 6. The acidic condition which is brought about by the decomposition of glucose, however, does not seem to pave the way for histamine formation.

From the above mentioned, the rôle of glucose seems far from being simple.

The increase of histamine formation which is attended with the bacterial growth can be classified into the following three stages: (1) Increase in the crops of bacterial cells which have a definite enzymatic activity. (2) Increase in the amount of the enzyme protein, or histidine decarboxylase per unit cell. (3) Congeniality of the environmental condition in culture media for the enzymatic reaction. The details of this problem concerning the effective substances, such as cyst(e)ine or redox potential-reducers and carbohydrates are beyond our comprehension, and more researches are necessary for the solution of the problem.

Summary

1. Various effects of several kinds of substance present in the culture media upon histamine formation attended with the growth of *Proteus morganii* have been studied, the complex medium consisting of the diluted solution of fish meat-extract and the synthetic medium being used.

2. None of those vitamin B complex including pyridoxal and pyridoxamine which we have tested promotes histamine formation.

3. Cystine, cysteine and methionine are strong in stimulating activity in the course of histamine formation. As the same phenomenon takes place by means of some other redox potential-reducers, this may be due to their power of reducing the redox potential of the media.

4. In the culture media which may be deficient in energy sources, the addition of glucose to it promotes histamine formation, and the degree of promotion becomes greater with the increased concentration.

5. Histamine can be far more produced by the combined action of cyst(e)ine and glucose than by their single one.

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Studies on the Strict Anaerobe Isolated from the Several Kinds of Swollen Canned Shell-fish

By

Masao KIMATA and Mikio AKAMATSU*

Recently, we were asked to examine swollen cans of several kinds of shell-fish (viz. boiled oysters, smoked oysters in oil and smoked baby clams in oil) which had been manufactured at a canned foods factory in Tohoku district in the spring of 1956. When the swollen cans were opened, the contents gave off an odor like human waste and looked sloppy as a mush. The microscopic examination of the contents showed bacteria of the prectridium form possessing terminaly a spore present in them. The contents looked differing from the usual swollen cannnd foods and rather resembled the case which had been reported by AMANO[1] for swollen canned oysters. AMANO isolated from them a high temperature resistant anaerobe belonging to the genus *Clostridium* and reported that this same species could always be found to exist in the intestines of fresh oysters as a normal flora. On the basis of this observation he emphasized the extreme care must be taken of the heat processing for the manufacture of canned shell-fish.

We also isolated an anaerobe belonging to the genus *Clostridium* from the swollen canned foods mentioned above. The work reported here deals with taxonomy of the isolated bacteria, the heat tolerance of its spores and the oxidation-reduction potential of the medium which may be responsible for the growth of the bacteria.

Experiments and Results

1. Samples.

Five swollen cans of boiled oysters, two of smoked oysters in oil and two of smoked baby clams in oil were used as the samples for the present experiments.

2. Isolation of bacteria.

All the swollen cans used as the samples were opened using the aseptic procedure. The bacteria concerned was isolated from their contents according to the methods described below. The isolation of aerobic bacteria was made as usual by the plate culture method with a glucose broth agar as the medium. The anaerobic bacteria was isolated anaerobically in BURRI tubes or WEINBERG tubes with a glucose broth agar containing vitamin C as the medium. Both cultures were incubated at 35°C. No colony developed on the aerobic plate cultures, but, for the anaerobic cultures the agar in the tubes cracked due to the formation of gas, which in turn made independent colonies hardly detectable. Thus we picked the colonies looked independent off the agar and inoculated them in a semisolid glucose broth agar medium containing vitamin C. The

* Laboratory of Microbiology

inoculated medium was incubated at 35°C. for 24 hours. After enrichment bacteria was isolated from the medium again using both BURRI tubes and WEINBERG tubes. This procedure was repeated several times to obtain the pure cultures.

3. Description and classification of the isolated bacteria.

All the pure culture isolated were examined for morphological, cultural and physiological characteristics by the usual methods. The results of the examinations showed that many respects of the characteristics of the strains isolated were practically the same from one another except for their abilities for the fermentation of rhamnose.

The description of the isolated bacteria is given below.

Morphology.

Rods : 1 to 2 microns by 3.5 to 5.0 microns. Straight with rounded ends, occurring singly. Motile with peritrichous flagella. Spore ellipsoid to oval, terminal, swelling rods. Gram positive.

Cultural characteristics.

Glucose agar colony : Irregular with undulate margin, smooth, moist, flat, glistening, translucent, grayish white.

Broth : Moderate turbid.

Glucose broth : Turbid with gas bubbles.

Litmus milk : Litmus was reduced, coagulated and peptonized after a week.

Physiological characteristics.

Cellulose was not decomposed. Indol was not formed. Nitrites were not produced from nitrates. Hydrogen sulfide was formed. Coagulated egg albumine was liquefied. Gelatine was not liquefied. Acids and gas were formed from glucose, sucrose and xylose. Gas was formed but no acids from arabinose, manitol, dextrin, glycerol and salicin. Fermentation of rhamnose varied with the strains. Lactose and raffinose were not fermented. Optimum temperature for the growth was 35°–40°C. No growth at 45°C. and 25°C. up to 48 hours. Optimum salinity for the growth was 2–3%. Strict anaerobic.

From these characteristics, this bacteria appears closely related to the organism which AMANO had isolated from the swollen canned oysters and reported to resemble *Clostridium fissum.*

According to the BERGEY's *manual 6th edition*[2], the form of the sporangia is an important key in the classification of the genus *Clostridium.* From this point of view this anaerobe may resemble *Clostridium sartagofornum* rather than *Clostridium fissum.* However, it rather differ from the former than the latter in other specific characteristics. The exact identification of the organism will be left for future research.

4. The heat-tolerance of the spores of the isolated bacteria.

After the isolated bacteria was cultured at 30°C, for three days in the glucose broth containing vitamin C, it was allowed to stand at room temperature for a week. Subsequently, the spores were harvested by centrifugation and washed three times with a phosphate buffer of pH 7.0 and resuspended therein. Then, the suspension was heated at 85°C. for five minutes in order to kill the vegetative cells. The suspension obtained in this way was put into ampoules of ca. 0.2 ml. capacity and sealed. The ampoules were heated at various given temperatures for definite intervals, and then

immersed in cold water. After cooling, their contents were put into the glucose broth containing vitamin C, and incubated at 35°C. for four days. The survival or the destruction of the spores was tested by the turbidity of the medium of the recovery cultures. Thus, the times necessary to kill the spores of the isolated bacteria were determined at various temperatures. The results obtained are shown in Table 1.

Table 1. Thermal death time of spores of the isolated bacteria in phosphate buffer (pH 7) at various temperatures.

Heating temp. °C.	Heating time (minutes)											
	30	35	40	45	60	75	90	100	240	260	280	300
100									+	+	±	−
105					+	+	−	−				
108-109	+	+	−	−								

+·····survived
−·····killed

As noted in the table, these spores were very resistant to heat ; they were killed respectively at 100°C. for 300 minutes, at 105°C. for 90 minutes and at 108–109°C. for 40 minutes in the phosphate buffer. The relation between the heating temperature and logarithm of thermal death time is represented by a straight line as shown in Fig. 1. The slope of this line gives a value of 10.9 for the temperature coefficient of the thermal death rate (Q_{10}).

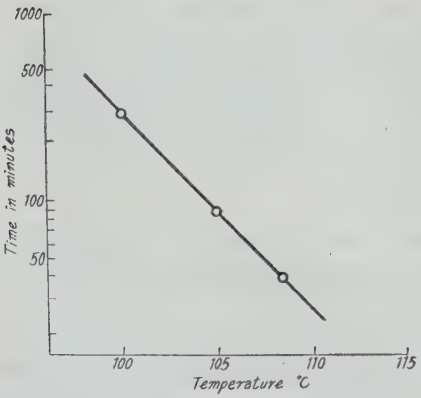

Fig. 1. Thermal death time of the spores.

5. *Oxidation-reduction potential of the medium.*

It is generally accepted that the growth of bacteria is closely related to the oxidation-reduction potential of the medium in which the growth takes place and that a particular bacteria has a certain definite range of the potential for the growth. Anaerobes which especially favour a reducing condition for the growth are not capable of multiplying in the medium having potential values higher than a certain limit.

Several attempts were made to determine a limiting potential value where anaerobes are unable to grow. However, even with the same anaerobic species, the potential values obtained were not always the same. It is considered that this is due to the differences in methods used for measurement of the potential and varieties of the kinds of the media and pH values in those experiments. KNIGHT and FILDES[3], working with *Cl. tetanii*, concluded that the limit potential for the growth was +0.110 volt at pH 7.2. PLOTZ and GELOSO[4] observed for *Cl. tetanii* a maximum oxidation-reduction

potential of +0.036 volt at pH 7.0. VENNESLAND and HANKE[6] found a positive limit of + 0.110 volt at pH 7.2 for *Bacteroides vulgatus*, and KLIGLER and GUGGENHEIM[6], working with *Cl. welchii*, found that the addition of vitamin C of 200 mg./1. brougt about the growth and gave a limit of -0.125 volt at pH 7.2.

Since the bacteria isolated by us from the swollen cans was strictly anaerobic, it is interesting to determine the limit potential for its growth. The following experiment dealed with this problem and the change in Eh value of the medium during the bacterial growth.

Procedures. Each of 100 ml. of glucose broth was put into a flask of 200 ml. capacity and sterilized as usual. The potentials of the medium were prepared by the addition of various concentrations of vitamin C. The concentrations of vitamine C added and the Eh values of the medium are recorded in Table 2.

Table 2. The initial oxidation-reduction potential of the glucose broth contaning various concentration of vitamin C.

Concentration of vitamin C (mg./1.)	0	40	80	300	1000	3000
Eh (volt)	+0.30	+0.23	+0.20	+0.18	+0.14	+0.09

The initial pH values of all the media were adjusted to 7.0. Each of the flasks was inoculated with two loopfuls of the bacteria which had been cultured in the broth at 35°C. A definite volume of the culture was taken out at definite intervals, and the potential and bacterial growth were measured. Concurrently, the potential of the medium in which the bacteria was not inoculated was determined as the control. The potential was measured by a potentiometer with a platinum electrode. The degree of bacterial growth was estimated from the optical density of the medium.

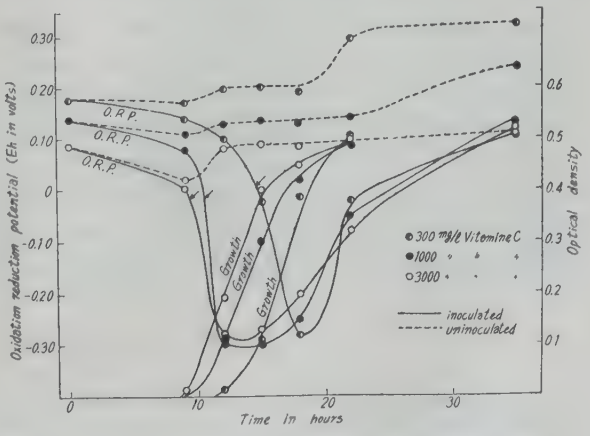

Fig. 2. The bacterial growth in the media containing different amonts of vitamin C and the change of Eh value in the media by the bacterial growth. The arrows in the figure indicate the Eh values at the beginning of the logarithmic growth phase.

Results. This anaerobe could not grow when the initial concentration of vitamin C in the medium was lower than 80 mg./1. When its concentration was higher than 300mg./1., the anaerobe grew well as illustrated in Fig. 2.

The potential of the medium drops down gradually in an early stage of the growth,

and when the Eh value reaches about zero volt, the logarithmic growth occurs accompanied with a rapid drop in the potential, and a maximum reducing potential of -0.30 volt appears in the retarded growth phase. The potential then begins to rise up again.

The pattern of the growth curve appears to be independent of the initial Eh value, provided it is so favourable as to allow growth of the bacteria. The lag phase of the growth tends to be slightly longer as the initial Eh value increases.

STOLP[7] found for *Cl. butyricum* that the potential of the medium dropped enough before the bacterial growth entered the logarithmic growth phase and reached a maximum reducing value at the beginning of this phase. The present experiment showed that the potential drop was somewhat delayed compared with that observed by STOLP.

In the medium in which the anaerobe was not inoculated, the potential slightly dropped in an early stage and then rised as seen in Fig. 2. This phenomenon may be due to the situation that the vitamin C in the medium was gradually oxidized and destructed by air penetrated into the medium during the course of incubation. The fact that low potential is kept longer as the initial concentration of vitamin C is increased may lend to support to this idia.

Discussion

Only one kind of anaerobic bacteria could be isolated from the swollen cans examined, but no aerobic one could be. Since no wrong was observed in the seaming part of all the cans used, it seemed that this organism was survived in the cans during heat processing. Although no bacterial examination could be made of the fresh materials and the sea water and the bottom muds with which these materials had been related, the fact that the isolated bacteria grew best in the media containing 3 % of NaCl appears to suggest that the organism was already present in sea water. Accordingly, the anaerobe may be originally found in the raw materials.

Our anaerobe is closely related to the organism which was isolated from swollen canned oysters by AMANO. He reported that it resembled *Cl. fissum*. However, our bacteria differs from *Cl. fissum* in respect of the location of the spore.

Table 3. The oxidation-reduction potential in several kinds of canned food.

Kinds of canned food	Vaccum (inch)	Eh (volt)
Boiled oyster	18	−0.07
Boiled chub mackerel	10	−0.08
Seasoned round herring	22	+0.01
Seasoned carrangoid	12	−0.03

A lot of liver or other reducing substances are contained in canned shell-fish such as oysters and baby clams. Therefore, the Eh values in these canned foods may be considerably low. From this fact canned shell-fish must be an especially good medium

for the growth of anaerobes. The potential of several kinds of canned foods were tentatively measured, and the results obtained are given in Table 3.

The samples examined were so limited that it is difficult to derive a definite conclusion from the data of this table. Either boiled oyster containing a lot of liver or raw-packed boiled chub mackerel shows a low potential, and moreover, even in the seasoned canned foods the potential is considerably lower than usualy expected. Therefore, as regards the potential canned shell-fish may not be better media than the other kinds of canned foods for the growth of anaerobes. Since this organism is able to grow when the Eh values of the media are lower than +0.18 volt, it may be one of the causative organisms for the swelling of general canned foods. Neverthless, it is accepted that only anaerobes can hardly cause the swelling of general canned foods, except for the case of canned shell-fish. This may be due to the fact that general canned foods are practically free from contamination of anaerobes.

Table 4. Thermal death time computed from Fig. 1.

Heating temperature		Time (minutes)
lbs.	°C	
4	106.9	56
5	108.4	40
6	109.9	28
7	111.3	20
8	112.7	15
9	113.9	11
10	115.2	8

The spores of this anaerobe are very tolerant to heat. The relation between the thermal death time and the heating temperature has been computed from the data given in Fig. 1 and is recorded in Table 4.

In general, the heat tolerance of bacterial spores depends considerably on the environmental conditions. Therefore, under practical condition used in the manufacture of canned foods, the thermal death times of the spores must be longer than those illustrated in Table 4. So, it seems quite difficult to keep swelling from canned shell-fishes, unless the central region of the can is heated sufficiently long.

The manufacturer of the cans used in this study informed us that they had been heated at 6 lbs for 60 minutes and that, on a dairy average, 3 % of the products swelled throughout the period of their manufacturing. The data of Table 4 indicates that the heat processing used by the manufacturer had been somewhat insufficient.

Summary

1. The anaerobe which is considered to be one of the causative organisms for the swelling of canned foods was isolated from the swollen cans of several shell-fish, viz., boiled oysters, smoked oysters in oil and smoked baby clams in oil.

2. This anaerobe was closely related to the organism which had been isolated by AMANO from the swollen cans of oysters.

3. The spores of the isolated anaerobe were very resistant to heat. They were destroyed at 100°C. for 300 minutes, at 105°C. for 90 minutes and at 108–109°C. for 40 minutes in the phosphate buffer of pH 7.0. The effect of the heating temperature on

the thermal death rate of the spores was expressed in the terms of the ARHENIUS equation and a temperature coefficient of 10.9 was obtained.

4. The isolated anaerobe was able to grow in the glucose broth containing vitamin C, and the limiting concentration for the growth was found to be 300 mg. per liter. The corresponding initial Eh value was measured and found to be +0.18 volt. A gradual fall in the potential occured accompanied with the start of the bacterial growth. The Eh value of about zero volt was observed at the beginning of the logarithmic growth phase independent of initial Eh values of the media.

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